UNIVERSITY OF ALBERTA

CHLORINE DIOXIDE INACTIVATION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN WATER

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA

SPRING, 1998

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: CHLORINE DIOXIDE INACTIVATION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN WATER submitted by: Lalith R.J. Liyanage in partial fulfillment of the requirement for the degree of DOCTOR OF PHOLOSOPHY IN ENVIRONMENTAL ENGINEERING.

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ABSTRACT

Chlorine dioxide was evaluated for its disinfection efficiency on *Cryptosporidium parvum* oocysts. Experiments were conducted in oxidant demand free phosphate buffered water using bench scale reactors. Preliminary evaluation of oxy-chlorine species for their disinfection effect, showed that free chlorine, chlorite, and chlorate had no disinfection effect on *C. parvum* at pH 8 and 22°C. Among the evaluated oxy-chlorine species, only chlorine dioxide was effective against *C. parvum*. These results suggested that chlorine dioxide is the active agent responsible for inactivation of *C. parvum* oocysts under typical conditions of chlorine dioxide application for drinking water disinfection

Chlorine dioxide was found to be significantly more effective in inactivating *C. parvum* oocysts at higher pH and at higher temperatures. Observed inactivation results were fitted to the Incomplete Gamma Hom (I.G.H) model, which can take chlorine dioxide disappearance into account. The I.G.H model was then used to compute concentration and contact time requirements for a given level of inactivation under given pH and temperature conditions.

Sequential disinfection with chlorine dioxide followed by free chlorine or monochloramine showed significantly more inactivation of oocysts due to synergism between the oxidants at pH 8 and pH 6 at 22°C. Also, the sequential treatment of oocysts by ozone followed by chlorine dioxide resulted in additional inactivation of *C. parvum* due to the synergism of the two disinfectants at pH 8 and 22°C.

ACKNOWLEDGEMENTS

The author is indebted to the University of Alberta, for the provision of University of Alberta Ph.D. scholarship. In addition, the author is grateful to the American Water Works Association and Chlorine dioxide Council of Chemical Manufacturers Association for the research grants provided through Dr. Gordon R. Finch.

The author wishes to thank his supervisor, Dr. Gordon R. Finch, who provided technical support throughout the research program. Also, the opportunities he made available to publish in variety of international forums and to meet leading figures in the field of environmental engineering are greatly appreciated.

The author wishes to thank Dr. Mike Belosevic (co-supervisor) and the members of examining committee for their valuable input during the research program.

The long discussions the author had with Dr. Lyndon L. Gyürék, contributed immensely to the successful completion of this work. Mr. Cezary Kucharski, Mr. Norman Neumann, Mr. Blair Falkner, Mr. Cori McHugh, Dr.(Mrs). Rebecca Guy, Mrs. Bhagya Liyanage, Mrs. Jolanta Kucharska, Ms. Daniella Weldon and Mrs. Persa Ceranic-Zagorac contributed in different ways to the conduct of laboratory works.

This work would have never been possible, without the patience and understanding of my wife, Bhagya Liyanage. Finally, I dedicate this work to my mother Seelawathie Liyanage, who never give up on encouraging me to study further.

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LIST OF SYMBOLS

C = chemical disinfectant concentration at time t, mg/L

C_t = chemical disinfectant concentration at the end of contact time, mg/L

C_o = initial chemical disinfectant residual at time zero, mg/L

d = estimated infectious dose per animal after disinfection,

d_o = initial number of oocysts per animal in the controls,

k = Hom inactivation rate constant,

$\hat{k}$ = optimal k parameter estimate,

k' = first-order chemical disinfectant decay rate constant, min^{-1},

L = likelihood function for normally distributed errors,

m = Incomplete gamma Hom model parameter,

$\hat{m}$ = optimal m parameter estimate,

N = number of surviving oocysts at time t,

N_o = number of oocysts at time zero,

n = Incomplete gamma Hom model parameter,

$\hat{n}$ = optimal n parameter estimate,

n = estimated infectious dose per animal after disinfection,

n_o = the number of oocysts given to each animal,

P = The proportion of the infected animal to cohort size for a given inoculum,

p = number of model parameters,

q = logit model predicted proportion of infected mice for the given cohort,

r = observed number of infected mice in the cohort,

T = total contact time, minutes,

T = Temperature, Kelvin

t = contact time, minutes,

u = cohort size (total number of mice inoculated with a given number of oocysts),

v = total number of experimental trials in a data set,

v_o = number of non-censored experimental trials in a data set,

X = the oocysts inoculum (log-units) given to each CD-1 neonate,

y_i = observed survival-ratio (log-units) for the i^{th} trial,

Y_i^c = infectivity detection limit for i^{th} trial,

α = level of statistical significance,

β = a set of parameters for a given model

β_o = intercept of the logit mean response model,

β_1 = slope of the logit mean response model,

χ = chi-squared distribution,

π' = logit mean response,

$\Phi(z)$ = standard cumulative normal distribution,

μ_i = Incomplete gamma Hom predicted survival ratio (log-units) for trial i

σ = square root of the maximum likelihood regression error variance,

γ = Incomplete gamma function,

Δt = time increment partition.

Subscripts

i = index of summation

Chapter 1

1. General introduction

1.1 OVERVIEW

For thousands of years, water has been known to cause diseases. However, the transmission of diseases by water was poorly understood until in 1854, John Snow showed that water supplied by a public water pump in Broad street, London, was the source of a cholera outbreak which claimed more than 500 lives (Snow 1855). Since then it has been found that a wide range of pathogenic micro-organisms are associated with transmission of diseases by water. In the late 1800s, it was learned that filtration could reduce turbidity, color and bacteria from drinking water. After the introduction of filtration to treat drinking water, epidemiological studies demonstrated that the incidence of cholera and typhoid fever was reduced dramatically. In 1912, liquid chlorine was first used to purify drinking water in Niagara Falls, NY (Faber 1952). By 1914, most of the drinking water supplied to cities in the United States was chlorinated in some manner. Since then chlorination continued to be an important barrier in preventing human exposure to pathogens in water.

However, in 1974, Bellar et al. (1974) and Rook (1974) showed that chloroform and other trihalomethanes (THM) were produced during chlorination of drinking water. Also subsequently U.S. Environmental Protection Agency (USEPA) surveys demonstrated that the trihalomethane were the organic chemicals occurring most consistently at probably the highest concentrations of any organic chemicals in treated drinking waters. Disinfectants used in water treatment and naturally occurring humic

substances in the source water were found as the most probable precursors to these compounds. These disinfection by-products are suspected to be carcinogens. Factors affecting the formation of disinfection by-products include the nature of the source water and content of precursor materials, the water temperature and pH, and conditions under which the disinfectant is used, such as the concentration, contact time, point of addition, and the residual maintained. Several options are available for the control of disinfection by-products (Craun et al. 1994). However, removing precursor compounds or removing by-products once they are formed can be both expensive and technologically complex for many water systems. Therefore alternative disinfectants, which minimize some of the known disinfection by-products, became attractive. However, it should be noted that, with the advancement of analytical tools and methods, more and more disinfection by-products will be identified in the future, making it a continuous endeavor to find better disinfectants.

On the other front, continuous increase in the demand for potable water and the concomitant pollution may increase the potential of more water-borne disease outbreaks. This fact together with the advancement of more and more tools available for disease surveillance has resulted in identification of number of recent disease outbreaks associated with drinking water. Since the 1980's encysted waterborne parasites such as *Giardia lamblia* and *Cryptosporidium parvum* have presented a challenge to water suppliers. It has been found that some waterborne disease outbreaks have been caused by either conventionally treated and disinfected drinking water supplies or by minimally treated water supplies meeting coliform bacteria, turbidity and other drinking water quality standards. During the past decade, *C. parvum* has emerged as one of the most

significant waterborne pathogens (Haas et al. 1996). The *C. parvum* oocyst has proven to be a difficult structure to penetrate using typical water disinfectants and are ubiquitous in surface waters (Galbraith 1989; LeChevallier et al. 1991; Ma et al. 1985; Ongerth and Stibbs 1987; Rose et al. 1988). As of now, *C. parvum* is known as the "super bug" within the drinking water industry and is targeted for stricter regulations and treatment guidelines designed to protect public health (Haas et al. 1996). Many studies have shown that chlorine, monochloramine and ultraviolet irradiation at practical plant doses are ineffective in controlling *C. parvum* oocysts (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987).

These findings of undesirable by-products of drinking water chlorination and relative inefficiency of chlorine in inactivation of encysted parasites have led to the study of alternative disinfectants. Among the alternative disinfectants for inactivation of *C. parvum* oocysts, preliminary studies indicated that ozone and chlorine dioxide are the most promising (Finch et al. 1993a; Korich et al. 1990b; Peeters et al. 1989).

1.1.1 Chlorine dioxide as an alternative disinfectant

Chlorine dioxide has number of advantages over ozone including: the ability to retain a residual over a longer period of time; better selective reactivity; and low production of halogenated organics (Miller et al. 1978; Rav-Acha et al. 1985). Chlorine dioxide can also be used for taste and odor removal, oxidation of soluble Fe, Mn, and sulfide (Aieta and Berg 1986). Another advantage of chlorine dioxide is that it is less corrosive and more compatible with construction materials (Kaczur and Cawlfield

1993). However, the major disadvantage of chlorine dioxide is that it is reduced to chlorite and chlorate under typical water treatment conditions (Aieta et al. 1984). Chlorite and chlorate are suspected of causing hemolytic anemia (Bull and Kopfler 1991).

1.1.2 Current understanding of *C. parvum* inactivation by chlorine dioxide

Only three studies have been reported on inactivation of *C. parvum* by chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993) with limited data. These studies mark the state of the art in understanding chlorine dioxide inactivation of *C. parvum*. However, these three studies reported only limited data and the effect of pH or temperature was not investigated. No studies have been done to evaluate the effects of major oxy-chlorine by-products, which are produced as inorganic by-products of aquatic reactions of chlorine dioxide or as unwanted by-products of chlorine dioxide generation, on the *Cryptosporidium* oocysts inactivation. No kinetic models were reported in literature for inactivation of *C. parvum* by chlorine dioxide. Therefore, there is a need for new data that will enable disinfection kinetic models to be developed. These models can then be used to evaluate different disinfection process design scenarios.

1.2 RESEARCH OBJECTIVES AND THE SCOPE OF STUDY

As a result of the above mentioned observations, goals of this study were set as:

1. to determine the efficacy of chlorine dioxide in inactivating oocysts under drinking water disinfection conditions.

2. to determine the effect of pH and temperature on the inactivation of *C. parvum* oocysts by chlorine dioxide.
3. to evaluate the combination of chlorine dioxide and other disinfectants in inactivating *C. parvum* oocysts.
4. to obtain design graphs for inactivation of *C. parvum* oocysts by chlorine dioxide which will aid engineers in evaluating chlorine dioxide inactivation of *C. parvum* in water.

To achieve the above goals, the following objectives were set and experiments were conducted in high quality laboratory water:

1. to investigate the effect of pH 6, and 11 in inactivating *C. parvum* oocysts by chlorine dioxide and to develop kinetic models
2. to investigate the inactivation of *C. parvum* oocysts by chlorine dioxide at 5 and 22°C and to develop kinetic models
3. to investigate the inactivation of *C. parvum* oocysts by sequential application chlorine dioxide and chlorine species at pH 8 and 22°C
4. to investigate the inactivation of *C. parvum* oocysts by sequential application of ozone and chlorine dioxide at pH 8 and 22°C and to develop kinetic models

All experiments were conducted in high quality oxidant demand free phosphate buffered water to remove water quality variables which may otherwise confound the desired outcome.

1.3 LITERATURE REVIEW

1.3.1 *Cryptosporidium*

Morphological analysis and cross-infection studies have indicated that there are at least four valid species of *Cryptosporidium* namely *C. parvum*, *C. muris*, *C. baileyi* and *C. meleagridis* (Robertson and Smith 1992). *C. parvum* is known as the species responsible for disease in humans. *Cryptosporidium* sp. including *C. parvum* has numerous animal hosts, including nearly 40 species of domestic and wild animals (Angus 1983).

The life cycle of *C. parvum* resembles that of other coccidia and has been well reviewed in a number of articles (Current 1987; Fayer and Ungar 1986; Robertson et al. 1994). Briefly, it completes its life cycle in a single host, and the most frequent location of infection is the gastrointestinal tract. Following excystation of ingested viable oocysts, sporozoites infect the epithelical cells at the luminal surface. Subsequent developmental stages have a unique, intracellular but extracytoplasmic location. In this parasitophorous vacuole, the sporozoite differentiates into the trophozoite, which then undergoes asexual multiplication. The schizonts formed develop into merozoites, which have the potential to invade proximal host cells, where they undergo further schizogony or initiate sexual multiplication. In sexual replication, the merozoites develop into either microgamonts or macrogamonts (male and female stages). Following fertilization, the macrogamont develops into an oocyst (Robertson and Smith 1992). The oocysts, the environmentally resistant stage of organism's life cycle, is excreted in large numbers by infected animals or humans. Oocysts are nearly spherical, with a diameter of around 5

μm. These oocysts are thick-walled and environmentally robust. Some oocysts with thin walls, may excyst before excreted and will thus continue the life cycle within the same host. This process is known as auto-infection.

1.3.1.1 The disease

Cryptosporidiosis is a diarrhoeal disease which is self-limiting in immunocompetent people, but has serious implications for the immunocompressed. Diarrhea may last a month or more but is self-limiting in otherwise healthy people. Abdominal cramping, vomiting, and fever are other common symptoms. The incubation period of cryptosporidiosis varies from two to twelve days, averaging about seven days. No drug is known to cure the disease, so that the recovery is dependent on the performance of the patient's immune system.

1.3.1.2 Routes of transmission

The number of oocysts in the environment is high because of the large number of infective oocysts excreted by hosts. Further, oocysts are robust and have the potential to survive for several months following excretion (Blewett 1989). Also oocysts were found to be resistant to most of the common disinfectants (Campbell et al. 1982) and survive longer in cold temperatures (Fayer and Nerad 1996; Robertson et al. 1992). Spread of the disease can result from contamination of drinking water supplies with the oocysts. However a number of other routes of transmission have been identified (Angus 1983; Robertson and Smith 1992) which include the person-to-person, animal-to-person and from contamination of the environment such as recreational use of water (Joce et al. 1991).

1.3.2 Treatment options to control *Cryptosporidium*

Assuming drinking water consumption of 2 L/person/day, it can be shown that the allowable number of oocysts in finished water is $3x10^{-5}$ oocysts/L to achieve the theoretical annual risk of 10^{-4} (1/10000 person) (LeChevallier and Norton 1995). Therefore depending on the level of the oocysts present in the raw water, water treatment utilities has to apply certain degree of removal/inactivation.

As a result, by controlling the oocysts concentration in the raw water, utilities can reduce the degree of removal/inactivation required by other water treatment processes and therefore should be considered as the first line of defense. *C. parvum* oocysts, which are present in the raw water may be removed or inactivated to control waterborne cryptosporidiosis. The following processes are considered the most important for removal of oocysts: coagulation, flocculation, sedimentation and filtration. Generally, disinfection is considered the last barrier which actually inactivates the oocysts.

Few studies have demonstrated that the level of human activity, both recreational and dairy farming, in watershed is directly related to the concentration of the oocysts in the river water (Hansen and Ongerth 1991; Roberts et al. 1995; Rose 1988) indicating the importance of watershed control as an option for controlling waterborne cryptosporidiosis. A rapid sand filter that receives water that has been properly pretreated is capable of achieving 3 or more log-units of removal of *Cryptosporidium*. On the other hand, inadequate pretreatment or poor filter operation may results in very low removal. Therefore, the importance in optimizing the coagulation, flocculation, and

sedimentation processes before filtration cannot be overlooked in controlling waterborne cryptosporidiosis.

Many studies have shown that chlorine, monochloramine and ultraviolet irradiation at practical plant doses are ineffective in controlling *Cryptosporidium* oocysts (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987). Findings by a number of research groups have led to the conclusion that ozone and chlorine dioxide are the most promising (Finch et al. 1993a; Korich et al. 1990b; Peeters et al. 1989). One of the most interesting recent findings indicates that, a greater inactivation can be expected when the combinations of disinfectants are used (Finch et al. 1995a).

Given the nature of the oocysts and the operational complexities involved with the unit operations of drinking water treatment, it can be concluded from the above discussion that, a single step in controlling *Cryptosporidium* is not advisable. On the contrary, multiple barrier approach, from watershed control, proper particle removal and adequate disinfection, should be considered to adequately protect consumers from waterborne cryptosporidiosis.

1.3.3 Interpretation of disinfection data

1.3.3.1 Viability assays

Unlike bacteria and viruses, *C. parvum* cannot be cultured outside a suitable host. Therefore an ongoing challenge in disinfection of *C. parvum* oocysts is the difficulty in defining the viability of the oocysts before and after disinfection. The ideal test for defining viability is the ability to cause disease in humans. However, this is not practical

and laboratory animals have been used as a surrogate. Animal infectivity is a difficult and tedious protocol relative to normal laboratory analyses in the water industry. Simpler methods have been sought including dye exclusion and in vitro excystation (Campbell et al. 1992b; Woodmansee 1987).

The excystation of oocysts constitutes a necessary step in the life cycle of *C. parvum* and normally occurs in the environment of the small intestine(Fayer and Ungar 1986). As with other coccidia, exposure to reducing conditions, enzymes and bile salts are all important factors of this process within the host (Korich et al. 1993). In *in-vitro* excystation, the oocysts are exposed to the conditions of the small intestine and induce oocysts to excyst artificially. Some of the drawbacks of in vitro excystation includes: 1) large number of oocysts are required for quantitative determinations; 2) there will likely be subjective discrimination of the percent excystation between different technicians; 3) it is not possible to determine whether the excysted sporozoite are able to complete the life cycle within a host.

When compared to other viability assays, vital dye staining is a rapid and convenient method for determination of protozoan viability (Taghi-Kilani et al. 1996). In vital staining techniques, different properties of live and dead oocysts and the response of a dye to one of these properties is assessed as the criterion for measurement of oocyst viability. For example, early studies using eosin were based on the ability of live oocysts to exclude the dye from the cell (Bingham et al. 1979; Chang 1944). Fluoresein diacetate (FDA) uses its ability to traverse cell membranes and to release fluorescein in viable cells. And propidium iodide (PI) which can only enters damaged cells which are considered to be non-viable. Number of other vital dyes are available in

the market today for *C. parvum*. Experimental studies using these dyes and *C. parvum* oocysts are less than convincing (Black et al. 1996; Campbell et al. 1992a; Campbell et al. 1997).

Currently, there is no absolute method for determining the proportion of an oocyst preparation that is infective. Hence, excystation correlation with infectivity is based on the underlying assumption that all of the oocysts that excyst were infective. This is not necessarily the case since excystation only determines the response of the oocyst to an external chemical stimulus, and provides no information about the potential for causing an infection in a susceptible host. The same problem occurs in *Giardia* studies (Labatiuk et al. 1991). Other studies have reported that in vitro excystation of *C. parvum* oocysts gave inconsistent results after ozonation (Finch et al. 1993a; Owens et al. 1994; Owens et al. 1995).

The underestimation of oocyst inactivation by in vitro excystation or vital staining (Finch et al. 1993a; Finch et al. 1995a; Finch et al. 1995b) has important implications for comparison of *Cryptosporidium* disinfection data collected in this study and reported in other studies.

While animal infectivity may be superior to in vitro methods for the time being, there are several sources of variation in the animal model system including the type and strain of animal used, the age of the animal, the infectivity of the oocysts, or the strain of *Cryptosporidium*. One study reported that of 19 strains of mice, only the adult beige mouse (C57BL/6J-bg) and the BALB/C neonates were susceptible to *C. parvum* after inoculation with 1×10^6 oocysts (Enriquez and Sterling 1991). The rat model does not

appear to be a sensitive measure of oocyst viability (Perrine et al. 1990) when compared with the neonatal mouse model.

The CD-1 mouse data from another study show that the CD-1 strain is susceptible to *C. parvum* and is an economical choice for experiments (Finch et al. 1994). However, the CD-1 model is not a surrogate for infection in humans and is strictly a tool for estimating the inactivation of oocysts even though the ID_{50} is similar to that reported in human volunteers (DuPont et al. 1995). All the data reported on this study is based on neonatal CD-1 mouse animal infectivity model described elsewhere (Finch et al. 1993b).

1.3.4 Effectiveness chemical disinfectants in inactivation of *Cryptosporidium*

1.3.4.1 Chlorine species

Chlorine is the most widely used chemical water disinfectant in the world. Early research in disinfection of *Cryptosporidium* reported that chlorine bleach solutions had little effect on oocyst viability (Campbell et al. 1982; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987). These studies often used chlorine bleach concentrations in 1,000's of mg/L to effect less than one log-unit inactivation. Another report suggested that free chlorine at practical plant doses (5 mg/L) was ineffective (Korich et al. 1990a). No animal infectivity data could be found in the literature for chlorine inactivation of *C. parvum* at doses (1 to 6 mg/L) and contact times (30 to 60 min.) that are practical for the water industry.

1.3.4.2 Ozone

Inactivation of *C. parvum* oocyst was studied by a team of USEPA scientists using an ozone pilot-plant to treat filtered Ohio River water (Owens et al. 1994; Owens et al. 1995). While the difficulties in this type of a study are significant, they were able to verify that the disinfection criteria that had been developed earlier at bench-scale (Finch et al. 1993a; Finch et al. 1994) was reasonable for ozone inactivation of *C. parvum* oocysts. Comparison of previous ozone disinfection studies involving *C. parvum* oocysts is somewhat subjective for three reasons: a suitable kinetic model has not been reported in each study; there is a potential disparity in viability measurements between animal infectivity and excystation; and the disinfection protocol varies from study to study. However, several studies indicated ozone as the most effective disinfectants against *C. parvum* oocysts (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993).

1.3.4.3 Chlorine dioxide

There have been few studies reported on the inactivation of *C. parvum* oocysts using chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). Only Korich et al. and Peeters et al. used animal infectivity as measures of post-disinfection oocyst viability. Ransome et al. used in vitro excystation.

Comparison among previous *Cryptosporidium* inactivation studies using chlorine dioxide is difficult because of the differences of viability assays used (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). Suitable kinetic models have not been reported for inactivation of *Cryptosporidium* oocysts by chlorine dioxide.

Peeters et al. worked with demineralized water at room temperatures and neutral pH. In their study, animal infectivity was used as the viability assay in which two to six days old neonatal Swiss OF1 mice were intragastrically inoculated and sacrificed for enumeration of oocysts production after 7 days of incubation. The experiments were conducted with 10^4 oocysts/mL and all the mice were given a dosage of 1 000 oocysts. The inactivation was estimated by comparing the oocyst production in the mice receiving treated oocysts with the production in the controls. This method assumes that oocyst production in the gut of infected mice is proportional to the viable number of oocysts present in the inoculum. *Cryptosporidium* oocysts are fully sporulated when passed into feces which are ready to make an infection as ingested and about 20% of the oocysts produced within the host are auto-infective. Each of these factors contributes to the possible heavy infection by even a small number of oocysts that may adversely affect the interpretation of the data. An alternative approach uses a dose-response model to estimate the number of infectious oocysts after disinfection (Finch et al. 1993b). Since Peeters et al. used a similar infectivity protocol to Finch et al., it is possible to crudely recalculate their data. The results are summarized in Table 1.1.

Another research group studied the effect of chlorine dioxide on *C. parvum* oocysts at pH 7 and 25°C (Korich et al. 1990a; Korich et al. 1990b). In vitro excystation was used as the main viability assay. Animal infectivity using 3 to 6 day old neonatal BALB/c mice were used to evaluate the viability for a few trials. Their inactivation data were not presented using the dose-response approach described by Finch et al. (1994). The ID_{50} that Korich et al. reported for the BALB/c mice was about 60 oocysts, similar to that of Finch et al. Consequently, a crude estimation of

inactivation can be made of Korich et al. data using the dose-response model of Finch et al. developed for CD-1 mice. These estimations from infectivity and the in vitro excystation data are summarized in Table 1.2.

The final study that is discussed here investigated the inactivation of *C. parvum* in spring water at pH 7 and 10°C using chlorine dioxide (Ransome et al. 1993). In vitro excystation was used to determine post disinfection viability of the oocysts. Their data are summarized in Table 1.3.

These three earlier studies mark the state of the art in understanding chlorine dioxide inactivation of *C. parvum*. Therefore, there is a need for new data that will enable disinfection kinetic models to be developed. These models can then be used to evaluate disinfection process design scenarios for use with the Enhanced Surface Water Treatment Rule (ESWTR) (U.S. Environmental Protection Agency 1994).

No studies have been done to evaluate the effects of major oxy-chlorine by-products, which are produced as inorganic by-products of aquatic reactions of chlorine dioxide or as unwanted by-products of chlorine dioxide generation, on the *Cryptosporidium* oocysts inactivation

1.3.4.4 Combination of disinfectants

There are number of other studies that reported the synergism between different disinfectants in inactivating bacteria and viruses. Kouame and Haas (1991) demonstrated that there is a synergistic interaction between free chlorine and monochloramine in killing *E.coli*. Katz et al., (1994) reported improved inactivation of fecal coliforms, fecal streptococci and *E. coliphages* when disinfected with chlorine

dioxide and free chlorine. It was also reported that sodium hypochlorite and methanol combination was shown to have enhanced effect in inactivating *Bacillus subtilis* spores (Coates and Death 1978; Gorman et al. 1983). Furthermore, a number of research groups have demonstrated copper and/or silver when applied with chlorine to have a synergistic effect in inactivating bacteria and viruses (Abad et al. 1994; Shuval et al. 1995; Yahya et al. 1992). In another study, chlorine and bromine solutions were found to be effective in inactivating chlorine resistant bacteria (Farkas-Himsley 1964). Ozone and hydrogen peroxide (Hall and Sobsey 1993), and ultraviolet irradiation and hydrogen peroxide (Bayliss and Waites 1979) combinations were also shown to demonstrate synergism.

Recent findings suggests that a similar synergistic effect can be observed with different disinfectants in inactivating *C. parvum* oocysts as well (Finch et al. 1995a). It was shown that shaking of *C. parvum* oocysts with sand was shown to increase the sensitivity of oocysts to the secondary application of chlorine which resulted in increased inactivation (Parker and Smith 1993). No studies were found which investigate the combinations of chlorine dioxide and other disinfectants in inactivating *C. parvum* oocysts.

1.3.5 Disinfection by-products of chlorine dioxide

Major reaction pathways of aqueous chlorine dioxide pertinent to drinking water disinfection are: oxidation, disproportionation and photolysis. Oxidation status of chlorine dioxide is pH dependent and the end product may be either chloride or chlorite:

$$ClO_2 + 4H^+ + 5e^- \rightarrow Cl^- + 2H_2O \qquad (1.1)$$

$$ClO_2 + e^- \rightarrow ClO_2^- \quad (1.2)$$

Transfer of 5 electrons occur only below pH 2 and hence is unlikely to be significant under typical water treatment conditions. On the other hand under neutral conditions (pH 7), chlorine dioxide accepts one electron resulting in chlorite as the end product. It has been found that 50 to 70% of applied chlorine dioxide is converted to chlorite under typical water treatment conditions (Aieta et al. 1984; Rav-Acha et al. 1985). Chlorine dioxide can also disproportionate via hydrolysis into chlorite and chlorate:

$$2ClO_2 + 2OH^- \rightarrow ClO_2^- + ClO_3^- + H_2O \quad (1.3)$$

Under ambient conditions the hydrolysis (Equation 1.3) is very slow and therefore considered to be insignificant under typical water treatment conditions (pH<10). However, hydrolysis can become rapid if chlorine or hypochlorous acid is present following the reaction, which predominantly forms chlorate in the pH 5-8 range:

$$2ClO_2 + HOCl + H_2O \rightarrow 2ClO_3^- + 3H^+ + Cl^- \quad (1.4)$$

At pH 6-8, chlorine dioxide disproportionate also to chlorous ($HClO_2$) and chloric ($HClO_3$) acids:

$$2ClO_2 + H_2O \rightarrow HClO_2 + HClO_3 \quad (1.5)$$

The rate of decomposition of chlorous acid varies with the pH, chlorite, chloride, ionic strength and temperature (Kieffer and Gordon 1968). However generally this equilibrium is shifted far to the left (Weber 1972) and is considered to be insignificant under drinking water disinfection conditions. In aqueous solutions, chlorine dioxide photolyzes in a complex manner. The principal stable end products are chlorate,

chloride, and hypochlorite(OCl^-) anions (Kaczur and Cawlfield 1993). However the extent of this reaction is considered to be insignificant under typical water treatment conditions (Aieta and Berg 1986).

The precursor material for chlorine dioxide production for drinking water treatment is sodium chlorite. The generation process can be broadly be divided into two processes based on the oxidation process: acid based process, and gaseous chlorine based process. Depending upon the pH, temperature, reaction time, quality and quantity of sodium chlorite, variable quantities of chlorine, chlorite and chlorate may be present in the end product.

1.3.6 Health effects of chlorine dioxide application

The health effects of chlorine dioxide can arise:

1. from chlorine dioxide or its metabolites,
2. from its inorganic by-products, and
3. from its organic reaction products.

1.3.6.1 Chlorine dioxide or its metabolites

The first direct evidence of chlorine dioxide toxicity was reported in 1955 (Haller and Northgraves 1955). They reported that chlorine dioxide increased mortality in rats at 100 mg/L level. Later Bercz et al. (1982) demonstrated that the chlorine dioxide caused a dose-dependent hyperthyroid activity (as noted by the reduction in serum thyroxin levels, which in turn involved with the control of neurobehavioral development). Interestingly the effects were observed only beyond a chlorine dioxide dose of 100 mg/L. Further, the chlorite or chlorate did not show any hyperthyroid

activity up to 400 mg/L. In another study, human volunteers were administered up to 5 mg/L of chlorine dioxide and were monitored for 84 days. The results of this study showed that chlorine dioxide did not cause any noticeable alterations in blood parameters, serum or urine chemistry values or any adverse physical symptoms (Lubbers et al. 1982).

The results of experiments done with animals so far show that there is a risk of hyperthyroid activities from chlorine dioxide at high concentrations. Limited studies done so far with humans (Michael 1981) have failed to show any significant adverse effect suggesting that normal healthy humans are unlikely to be affected at the concentrations usually encountered in drinking water disinfection. However more studies are needed to verify the safety of individuals with impaired thyroid functions receiving such waters and the long term effects on healthy individuals.

1.3.6.2 Inorganic by-products

Chlorite and chlorate are the main inorganic by-products of chlorine dioxide and are the most extensively studied species of chlorine dioxide for their toxicological properties. Because these ions are suspected of causing hemolytic anemia (Chlorite is considered to be the most potent among the two). Hemolytic anemia is characterized by the appearance of brownish or greenish derivatives of hemoglobin including methemoglobin granules called Heinz Bodies within the red blood cells. A series of studies were triggered by the findings of (Musil et al. 1963) that chlorite and chlorate were capable of producing methemoglobinemia in vitro. This study was confirmed later by a study done in-vitro with human, rat and guinea pig bloods (Heffernan et al. 1979).

However in-vivo studies (Cohen et al. 1964) done with rats did not show significant reduction in red blood cell counts even at the concentration level of 100 mg/L of chlorite. There were some reduction in hemoglobin at chlorite concentration of 100 mg/L but hemoglobin level fully recovered after 90 days of exposure. As for the case of hyperthyroid activity of chlorine dioxide, the effect of chlorite ion probably not cause hemolytic anemia in normal humans at the concentrations normally encounter in drinking water disinfection (Bull 1982). However there is concern for those individuals who have exceptional sensitivity to hemolytic agents.

1.3.6.3 Organic by-products

Chlorine dioxide reacts primarily by oxidation-reduction reactions (as opposed to chlorine, which reacts not only via oxidation but also by electrophilic substitution) resulting in few chlorinated organic compounds. Chlorine dioxide also appears to be more selective or less reactive in typical water treatment applications (Aieta and Berg 1986). It does not produce THM in reaction with both humic and fulvic acids but other organic halides (TOX) can be formed. However only 1-25 % of the TOX is formed with chlorine dioxide compared to that with chlorine under comparable conditions (Chow and Roberts 1981; Fleischacker and Randtke 1983; Symons 1981). The presence of bromide ions in water can result in significant levels of brominated haloforms which of greater toxicological concern (Lange and Kawczynski 1978).

The organic reaction products of chlorine dioxide with phenol includes chlorophenols, *p*-benzoquinone, and malaic and oxalic acids (Masschelein 1979; Symons 1981; Wajon et al. 1982). The product distribution is dependent on the chlorine

dioxide to phenol ratio, and under normal conditions of drinking water disinfection, major organic products that would be expected are *p*-benzoquinone and in addition to the low molecular weight aldehydes and carboxylic acids (Aieta and Berg 1986). The evidences accumulating so far suggest that under normal conditions, chlorine dioxide treatment of drinking water does not pose acute or chronic health effects resulting from its organic by-products and these products do not exhibit significant indications of mutagenic or carcinogenic activities, unless significant amount of bromide ion is present in water (Aieta and Berg 1986).

1.3.7 Reduction of oxy-chlorine species in drinking water

The use of granular activated carbon (GAC), use of sulfur-based reducing agents and use of ferrous ions have been studied to remove chlorite and chlorate ions.

Sulfur dioxide and sulfite ions(SO_2/SO_3^{2-}) were shown to be effective in removing chlorite ions (Gordon et al. 1990). The end products were sulfate ions and chloride:

$$2SO_3^{2-} + ClO_2^- \rightarrow 2SO_4^{2-} + Cl^- \qquad (1.6)$$

This study was conducted in the absence of oxygen in the reactor system. In the presence of dissolved oxygen, however, in addition to the sulfate ions and chloride ions, chlorate ions are also formed (Dixon and Lee 1991; Griese et al. 1991):

$$ClO_2^- + SO_2 \rightarrow O_2S - ClO_2^- + O_2 \rightarrow SO_3 + ClO_3^- \qquad (1.7)$$

Under the drinking water treatment conditions, dissolved oxygen will be present invariably making sulfur dioxide and sulfite ions(SO_2/SO_3^{2-}) method unacceptable under some conditions. On the other hand, sodium thiosulfate ($Na_2S_2O_3$) was shown to remove chlorite ions effectively with little or no chlorate formation (Griese et al. 1991). The end

products presumably are the sulfate ions and chloride. Dissolved oxygen did not seem to interfere with this reaction. However, the degree of chlorite reduction greatly depend on sample pH and contact time (at pH 6.1 it took 120 min. to reduce chlorite 4 mg/L to 1 mg/L with 40 mg/L of sodium thiosulfate). Considering these facts it can be concluded that even though sodium thiosulfate favors over the sulfur dioxide and sulfite ions, removal of chlorite ions using sodium thiosulfate may not be attractive for drinking water industry.

The utilization of ferrous has shown to be promising for removal of chlorite ions at the pH range of 5.5-8.0 (Griese et al. 1991; Iatrou and Knocke 1992) with no significant formation of chlorate:

$$4Fe(II) + ClO_2^- + 10H_2O \rightarrow 4Fe(OH)_3(S) + Cl^- + 8H^+ \qquad (1.8)$$

This reaction appeared to be complete within few minutes. No adverse effect from dissolved oxygen was observed. Ferrous ion reduction process had no effect on existing chlorate ions. However in a later study by same authors (Griese et al. 1992) showed that localized low level pH conditions resulting from the ferrous ion reduction process may contribute some chlorate ions to the system. Application of granular activated carbon (GAC) does not seems to be an economical solution (Dixon and Lee 1991) for removing chlorite ions. It has further shown that granular activated carbon had very little affinity for chlorate ions except for the earliest stages of bed life. Therefore, it can be concluded that the only viable technology for chlorite removal is the application of ferrous ion reduction. However, there is no known technology that can remove chlorate ions from drinking water.

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Table 1.1. Estimated inactivation of *C. parvum* oocysts at room temperature in buffered laboratory water.

Initial ClO_2 residual (mg/L)	Final ClO_2 residual (mg/L)	Contact time (min.)	Inactivation[1] (%)
0.31	N/R[2]	8	82
0.31	0.07	16	82
0.43	N/R	15	90
0.43	0.22	30	91

1. Animal infectivity (dose response estimated from CD-1 mouse model
2. Not reported

Source: After (Peeters et al. 1989)

Table 1.2. Estimated inactivation of *C. parvum* oocysts at pH 7 and 25°C in buffered laboratory water.

Initial ClO_2 residual (mg/L)	Final ClO_2 residual (mg/L)	Contact time (min.)	Inactivation (%)	
			Infectivity[1]	Excystation
0.6	<0.2	15	N/D[2]	17
	N/R	30	N/D	17
	N/R	45	N/D	22
	N/R	60	N/D	42
1.3	N/R	15	N/D	31
	N/R	30	<90	76
	N/R	45	99	93
	0.4	60	99	95

1 Estimated using CD-1 mouse dose-response model (Finch et al. 1994)
2 No data
3 Not reported

Source: After (Korich et al. 1990a; Korich et al. 1990b)

Table 1.3. Inactivation of *C. parvum* oocysts at pH 7 and 10°C in spring water.

Initial ClO2 residual (mg/L)	Final ClO2 residual (mg/L)	Contact time (min.)	Inactivation[1] (%)
0.46	0.41	15	28
0.49	0.43	30	64
5.0	4.0	15	96

1. In vitro excystation.

Source: (Ransome et al. 1993)

Chapter 2

2. Effect of aqueous chlorine and oxy-chlorine compounds on *C. parvum oocysts*[1]

2.1 INTRODUCTION

Relative inefficiency of conventional disinfectants in inactivation of encysted parasites (Campbell et al. 1982; Korich et al. 1990; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987) has led to the study of more powerful alternative oxidants for drinking water disinfection. Among the alternative disinfectants, chlorine dioxide is promising (Finch et al. 1995; Peeters et al. 1989). It has been reported that 50 to 70% of applied chlorine dioxide is converted to chlorite under typical water treatment conditions (Aieta et al. 1984; Rav-Acha et al. 1985; Werdehoff and Singer 1987). Some percentage of these chlorite ions may be reduced further to chloride ions depending on the contact time and water quality. Hydrolysis of chlorine dioxide into chlorite and chlorate is slow under drinking water disinfection conditions, unless chlorine or hypochlorite ions are present (Aieta and Berg 1986; Taube and Dogden 1949). The pre-cursor material for chlorine dioxide production for drinking water treatment is sodium chlorite. Chlorine dioxide is generated by the reaction of aqueous chlorine and sodium chlorite. Depending upon the pH, temperature, reaction time, quality and quantity of sodium chlorite, variable quantities of aqueous chlorine, chlorite and chlorate may be present in the end product. Therefore, it can be

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[1] A version of this chapter is Published in Environmental Science and Technology (1997); Vol. 31, p 1992-1994

concluded that under the conditions of drinking water disinfection with chlorine dioxide, the major species of interest would be chlorine dioxide, chlorine, chlorite, chlorate and chloride. Very little is known about the effect of chlorine dioxide by-products on the inactivation of microorganisms (Noss and Olivieri 1985). No studies have been found that investigate the effect of these by-products on *C. parvum* oocysts.

The primary goal of this study was to determine the effect of chlorine dioxide disinfection by-products on inactivation of *C. parvum* oocysts. In the present study, effect of chloride was not evaluated considering the insignificance of contribution of chloride from chlorine dioxide application compared to the background chloride concentrations naturally found in water.

In laboratory experiments (which are designed to quantify the effect of chlorine dioxide on *C. parvum* oocysts), at the end of the chlorine dioxide contact time, remaining residual chlorine dioxide is neutralized using sodium thiosulfate. However, at the end of the chlorine dioxide contact time, chlorite and chlorate are also present in the reaction vessel. Therefore, it is important to find out the effect of simultaneous application of sodium thiosulfate with chlorite or chlorate on *C. parvum* oocysts to distinguish the effect of chlorine dioxide and by-products of sodium thiosulfate and chlorite or chlorate on oocysts. Therefore, experiments were also conducted to discover the effects of simultaneous application of sodium thiosulfate and chlorite or chlorate on *C. parvum* oocysts.

2.2 MATERIALS AND METHODS

2.2.1 Parasite methods

At the end of each experimental trial, the test reactor and control vessels were analyzed for infectious oocysts using an animal dose-response model described elsewhere (Finch et al. 1993b). Details of parasite methods are provided in Appendix B. The log inactivation was estimated from:

$$\log\left(\frac{N}{N_0}\right) = \log\left(\frac{n}{n_0}\right) \qquad (2.1)$$

where: n is the estimated infectious dose per animal after disinfection, n_o is the initial number of infectious oocysts in the controls, N and N_0 are the number of infectious oocysts before and after disinfection respectively. Details of the *C. parvum* methods, including production of oocysts, concentration of samples, inoculation into the animal host, and subsequent determination of infection have been described previously (Finch et al. 1993b) (also details are provided in Appendix B) with the addition of an improved oocyst concentration method where a cesium chloride gradient is used rather than a sucrose gradient (Kilani and Sekla 1987). The net survival ratio for each experimental trial was calculated by subtracting the survival ratio calculated for the positive control from that of the chemical disinfectant treated group.

2.2.2 Chlorine dioxide generation

Chlorine dioxide stock solution was generated by a patented chlorine dioxide generator (CDG Technology, Inc., New York, NY) where dilute chlorine gas in a nitrogen carrier is passed through a packed bed of sodium chlorite. The gas flow rate

was adjusted so that all of the chlorine gas was consumed in the reaction column. The process gas was captured in a 500 mL gas absorption flask containing Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm). The concentration of free chlorine, chlorate and chlorite were determined using amperometric titration (Greenberg et al. 1992).

2.2.3 Preparation of chlorine, chlorite and chlorate

Free chlorine stock solutions of approximately 600 mg/L were prepared daily as needed using sodium hypochlorite solution (6% available chlorine, BDH Inc., Poole, England) with oxidant demand free water. Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm) was made oxidant demand free following previously published procedures (Finch et al. 1987) as described in Appendix A. Analytical reagent grade sodium chlorite and sodium chlorate (AnalaR grade, BDH Inc., Poole, England) were used to prepare stock solutions in oxidant demand free water immediately prior to the experiments.

2.2.4 Disinfection procedure

The disinfection procedures used in this study were similar to those used in earlier work with ozone (Finch et al. 1993a). The details of disinfection procedures are provided in Appendix A. Experimental protocols followed those described elsewhere for chlorine dioxide (Haas et al. 1993). Experiments were conducted on a bench top in a temperature controlled room. Room temperature was measured to be 22±1°C throughout the experiment. A fresh working stock solution of chlorine dioxide was prepared before each experiment from the stored generated chlorine dioxide stock. The

test preparation of 5×10^6 oocysts was suspended in 40 mL of oxidant demand-free, 0.05 M phosphate buffer (pH 8) in 50 mL Teflon® coated Nalgene® (Nalge Co.,Rochester, NY) reactors. A measured volume from the chlorine dioxide working stock solution was added to the reactor using a calibrated pipette and stirred using a platform shaker (Benchtop Shaker Model G33, New Brunswick Scientific, Edison, NJ) at 100 rpm. The concentration of chlorine dioxide in aqueous solution was continuously monitored using ultraviolet spectrophotometry at 360 nm using a molar absorptivity of 1250 M^{-1} cm^{-1} (Gordon et al. 1992). At the end of the contact time, residual chlorine dioxide was neutralized with 0.1 N sodium thiosulfate prepared from reagent grade sodium thiosulfate (AnalaR grade, BDH Inc., Poole, England).

The stock chlorine solution was stored in dark refrigerated conditions during the day of the experiment. Chlorine concentration of the working stock was determined by forward amperometric titration or by the DPD (N,N-diethyl-*p*-phenylenediamine) procedure (Greenberg et al. 1992). A measured volume from the chlorine working stock solution was added to the reactor using a calibrated pipette and stirred using a platform shaker (Benchtop Shaker Model G33, New Brunswick Scientific, Edison, NJ) at 100 rpm. After the desired contact time, residual chlorine was neutralized with 1 M sodium sulfite made from reagent grade sodium sulfite (AnalaR grade, BDH Inc., Poole, England).

2.3 RESULTS AND DISCUSSION

The results of the experiments with chlorine dioxide, aqueous chlorine, sodium thiosulfate chlorite, and chlorate are summarized in Table 2.1. Chlorine dioxide was an

effective disinfectant under the experimental conditions of this study. There was no appreciable effect from either aqueous chlorine, sodium thiosulfate, chlorite or chlorate when applied alone, on inactivation of *C. parvum* oocysts at the levels applied. The data suggest that the inactivation of *C. parvum* oocysts was associated only with chlorine dioxide, not the results of chlorine or disproportionation products of chlorine dioxide. These results are consistent with previous findings (Noss and Olivieri 1985) that chlorine dioxide was effective in inactivation of *f2* virus whereas there was no noticeable viricidal activity of chlorite, chlorate or chloride ions. The results of the present study indicate that increased efficacy of chlorine dioxide observed, in another study, at higher pH for *Giardia* (Leahy 1985) may be attributed to the chemical or physical changes in *Giardia* cysts structure and not due to the increased disproportionation of chlorine dioxide at higher pH.

Experimental results of simultaneous application of sodium thiosulfate and chlorite or chlorate (Table 2.2) indicate that the simultaneous application of chlorite or chlorate with sodium thiosulfate had no effect on *C. parvum* oocysts. Implications of this results are that, for disinfection studies with chlorine dioxide, use of sodium thiosulfate to neutralize residual chlorine dioxide at the end of contact time does not confound the inactivation of oocysts attributed to chlorine dioxide alone. Under the conditions of this study, presumably, sodium thiosulfate does not react with chlorate but will react with chlorite to produce sulfate ions and chloride ion (Griese et al. 1991). Therefore, the results reported in Table 2.2 indirectly suggest that sulfate ions or sodium thiosulfate itself has no inactivation effect on *C. parvum* oocysts.

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Table 2.1. Effects of aqueous chlorine and oxy-chlorine compounds on *C. parvum* oocysts inactivation at pH 8 and at 22±1°C

Compound	Initial residual concentration, mg/L	Contact time, min.	Inactivation ratio, log-units
Chlorine dioxide	3.3	116	>3.22
Chlorine dioxide	2.0	61	1.57
Chlorine dioxide	2.0	30	0.99
Aqueous chlorine	4.0	240	0.10
Sodium thiosulfate	9.1*	1063	0.02
Chlorite	4.0*	730	0.00
Chlorate	4.0*	730	0.07

* Applied concentration

Table 2.2. Effect of simultaneous application of sodium thiosulfate and chlorite or chlorate on *C. parvum* oocysts inactivation at pH 8 and at 22±1°C

Mixture of compounds	Applied concentration A, mg/L	Applied concentration B, mg/L	Contact time, min.	Inactivation ratio, log-units
Chlorite + Sodium thiosulfate	4.0	9.1	548	0.0
Chlorate + Sodium thiosulfate	4.0	9.1	543	0.0

Chapter 3

3. Effect of pH and temperature on inactivation of *C. parvum* by chlorine dioxide[1]

3.1 INTRODUCTION

C. parvum is a coccidian parasite of human and many animal species. The parasite is transmitted by ingestion of oocysts that have been excreted in the feces of infected humans or animals. The infection can be transmitted through person-to-person or animal-to-person contact, ingestion of fecally contaminated food or water. Waterborne cryptosporidiosis outbreaks have been documented in Canada, the United States and Great Britain (Gallaher et al. 1989; Hayes et al. 1989; MacKenzie et al. 1994; Pett et al. 1994; Richardson et al. 1991). Many studies have shown that chlorine, monochloramine and ultraviolet irradiation at practical plant doses are ineffective in controlling *C. parvum* oocysts (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987). Among the alternative disinfectants, ozone and chlorine dioxide are the most promising (Finch et al. 1993; Peeters et al. 1989).

Chlorine dioxide has number of advantages over ozone including: the ability to retain a residual over a longer period of time; better selective reactivity; and low production of halogenated organics (Miller et al. 1978; Rav-Acha et al. 1985).

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[1] A version of this chapter is submitted for review in the *Journal of Environmental Engineering*, ASCE

However, there have been only few studies reported on the inactivation of *C. parvum* oocysts using chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). These three earlier studies mark the state of the art in understanding chlorine dioxide inactivation of *C. parvum*. Therefore, there is a need for new data that will enable disinfection kinetic models to be developed. No studies were found which address the effect of pH and temperature on inactivation of *C. parvum* oocysts by chlorine dioxide.

The objective of this study is to evaluate the effect of pH and temperature on inactivation of *C. parvum* oocysts by chlorine dioxide, and also to present the kinetic models that can be used to generate chlorine dioxide process design requirements for controlling *C. parvum* in drinking water.

3.2 MATERIALS AND METHODS

Chlorine dioxide generation is described in Section 2.2.2

3.2.1 Disinfection procedure

Details of the disinfection procedure is given in Appendix A. The results presented in Chapter 2 was obtained using 50 mL Nalgene reactors with $5x10^6$ oocysts in each reactor. Also, generally the cohort size (number of mice used) were limited to five. However, to obtain the results presented in this study, the test preparation of $10\text{-}20 \times 10^6$ oocysts was suspended in 200 mL of oxidant demand-free, 0.05 M phosphate buffer. The reactor vessel was a 250 mL Erlenmeyer flask. All reactors were made ozone demand-free prior to use by methods described elsewhere (Finch et al. 1994). A measured volume of chlorine dioxide from a concentrated stock solution was added to

the reactor, and reactor content was agitated using a Teflon®-coated magnetic stir bar. The reactor (containing oocysts) was continuously sampled in a closed loop at a flow rate of 8 mL/min. The sample was carried through a short piece of small diameter Teflon® tubing to a 35 µl flow cell with a light path of 1 cm and the concentration of chlorine dioxide in aqueous solution was continuously monitored using ultraviolet spectrophotometry at 360 nm using a molar absorptivity of 1,250 $M^{-1} \cdot cm^{-1}$ (Gordon et al. 1992). At the end of the contact time, residual chlorine dioxide was neutralized with sodium thiosulfate.

3.2.2 Estimation of inactivation ratio

Animal infection procedure is described in Section 2.2.1. and in Appendix B. The estimated infectious dose was calculated from a logistic dose-response model developed for each individual batch of oocysts. Oocysts obtained from each experimentally infected neonatal calf was regarded as an individual batch of oocysts. The logit mean response is given by (Neter et al. 1989):

$$\pi = \ln \frac{P}{1-P} = \beta_o + \beta_1 X \qquad (3.1)$$

where P is the proportion of the cohort positive for a given inoculum X (log-units) and β_o and β_1 are the logit response model parameters. Model parameters were estimated using the method of maximum likelihood and the solver function of Excel 5.0 (Microsoft Corp.). Maximum likelihood estimators of β_o and β_1 are those values that maximize the natural logarithm of the likelihood function (Brand et al. 1973; Cox 1970):

$$\ln L = \sum_{i=1}^{a} Y_i(\beta_o + \beta_1 X_i) - \sum_{i=1}^{a} \ln\{1 + \exp(\beta_o + \beta_1 X_i)\} \qquad (3.2)$$

where $Y_i = 0, 1$; and $i = 1, 2, \ldots,$ up to a, the number of neonate CD-1 mice used in the dose-response study for a particular batch of oocysts. This approach gives the reduction of viability of experimental results relative to dose-response trials independent of the viability of the stock oocysts seeded in reactors. It was observed that the viability of stock oocysts for each batch of oocysts decreased over the time. Therefore, to minimize bias caused by decreasing stock oocyst viability, dose-response trials were spread over a 1 to 3 month usage period for each batch of oocysts. The net survival ratio for each experimental trial was then calculated by subtracting the survival ratio calculated for the positive control from that of the treated group. This accounts for loss of infectivity that may occur over time.

3.2.3 Goodness of fit

After a logistic dose response model has been fitted, the departures of the observed responses from those calculated from the fitted line was assessed by the Pearson chi-square test. For this, the Pearson chi-square statistic (Equation 3.3) was calculated (Hewlett and Plackett 1979).

$$\chi^2 = \sum \frac{(r - uq)^2}{uq(1-q)} \qquad (3.3)$$

where, r is the observed number of infected mice, u is the cohort size and q is the predicted proportion of infected mice. The value of X^2 is referred to a table of chi-square, with degrees of freedom equal to the number of dose levels less number of

parameters (two in this case). If the probability arrived at is greater than 0.05, the fit of the observations to the logistic line is regarded as adequate.

3.2.4 Kinetics of disinfection

Typically, chemical disinfection results have been expressed in terms of a concentration x time product (CT) for different level of inactivation (Hoff and Akin 1986). The CT product can be derived theoretically from the Chick-Watson, pseudo first-order rate law (Chick 1908; Watson 1908):

$$\log\left(\frac{N}{N_0}\right) = -kC^nT \tag{3.4}$$

where k is a reaction rate constant found experimentally; N_0 and N are the number of survivors initially and after the contact time T respectively, C is the concentration of the disinfectant; n is an empirical constant (or coefficient of dilution).

An alternate model has been proposed (Hom 1972) to account for deviations from the Chick-Watson pseudo first-order rate law observed in practice:

$$\log\left(\frac{N}{N_0}\right) = -kC^nT^m \tag{3.5}$$

where *m* is an empirical constant. Like the Chick-Watson, Hom's model assumes that the chemical disinfectant concentration, C, remains constant.

In order to, more accurately, define the relationship between chemical disinfectant concentration and predicted log-survival of microbes, a Hom-type model can be modified to account for decreasing disinfectant residuals resulting integral-Hom model described previously (Finch et al. 1997):

$$\log\left(\frac{N}{N_0}\right) = -kmC_0^{\,n}\int_0^T e^{-k'tm}t^{m-1}\,dt \qquad (3.6)$$

where k' is the first-order disinfectant decay rate constant and C_0 is the initial disinfectant residual. The equation 3.6 can be solved numerically using equation 3.7, with solver routine of Microsoft® Excel 5.0(Microsoft Canada. Mississauga, Ontario) to maximize lnL function as described in next section.

$$\log\left(\frac{N}{N_0}\right) = -kmC_0^{\,n}\sum_{x=1}^{Z}(e^{-k't})_x{}^n t_x{}^{m-1}\Delta t_x \qquad (3.7)$$

where z is the total number of partitions, x is the index of summation and Δt is the time increment partition, the cumulative sum of which equals the disinfectant contact time, T.

It has been shown that (Haas and Joffe 1994), a closed form of Hom model can be derived using the Incomplete Gamma function (Deming 1944):

$$\gamma(\alpha, x) = \int_0^x e^{-z}Z^{\alpha-1}dz \qquad (3.8)$$

where $\alpha>0$, and $x\geq 0$

The final solution can then be expressed in the following form:

$$\log\frac{N}{N_0} = \frac{-mkC_0^n}{(nk')^m}\cdot\gamma(m, nk't) \qquad (3.9)$$

where $m>0$. $nk't\geq 0$

If a table of the incomplete gamma function is available, the explicit survival ratio can be computed. Gamma function available in Microsoft® Excel (Microsoft Canada, Mississauga, Ontario) together with solver routine was used to calculate the model parameters in this study.

In the present study, it has been assumed that chlorine dioxide decomposition follows first-order kinetics. This appears to be a reasonable assumption based on our observations. However, this assumption remains to be verified over a wide range of water qualities. The first order decay rates were calculated using measured residual chlorine dioxide concentrations (at least 6 observations) during the contact time of each experiment. The solver routine of Microsoft® Excel 5.0 (Microsoft Canada, Mississauga, Ontario) which uses the conjugate gradient method (Powell 1965), was used to minimize the error function to calculate the first order decay rate.

3.2.5 Temperature dependence of reaction rates

In general, the rates of most chemical and biological reactions increase with temperature. The change in rate constant with temperature can be expressed mathematically by the Arrhenius equation:

$$\frac{d(\ln k)}{dT} = \frac{E_a}{RT^2} \tag{3.10}$$

where $d(\ln k)/dT$ represents the change in the natural log of the rate constant with temperature, R is the universal gas constant, and E_a is a constant for the reaction termed the activation energy. Integrating between limits gives:

$$\ln\left(\frac{k_2}{k_1}\right) = \frac{E_a(T_2 - T_1)}{RT_2T_1} \tag{3.11}$$

where k_2 and k_1 are the rate constants at temperatures T_2 and T_1 respectively. Temperature is expressed in kelvin.

Another common form of the equation describing temperature dependence is

$$\frac{k_2}{k_1} = \theta^{(T_2 - T_1)} \qquad (3.12)$$

where θ is considered a constant for over a small temperature ranges near ambient temperatures.

3.2.6 Experimental design

The experimental settings for concentration and contact time were determined using the Box-Lucas criterion (Box and Lucas 1959) which provides experimental settings (concentration and contact times in this case) such that model parameters for Hom-type models can be estimated efficiently. Inactivation kinetics for chlorine dioxide at pH 6 and 22°C, were modeled using 7 trials. Initial residual concentration of chlorine dioxide ranged from 0.5 to 4.5 mg/L and contact times from 30 to 179 minutes (Table 3.2). Inactivation kinetics for chlorine dioxide at pH 11 and 22°C, were modeled using 8 trials. Initial residual concentration of chlorine dioxide ranged from 0.4 to 4.0 mg/L and contact times from 30 to 180 minutes (Table 3.3). Inactivation kinetics for chlorine dioxide at 5°C and pH 6 were modeled using 9 trials. Initial residual concentration of chlorine dioxide ranged from 0.6 to 10.8 mg/L and contact times from 30 to 362 minutes (Table 3.4).

3.2.7 Parameter estimation

Kinetic parameters were estimated using maximum-likelihood. Maximum-likelihood provides parameter estimates with many desirable statistical properties and also allows the use of censored data. Details of the technique and the statistical properties of the estimated parameters are described elsewhere (Haas and Heller 1989;

Haas and Jacangelo 1993). However the major disadvantage is that this methodology requires strong assumptions about the structure of the data. The kinetic parameters k, m, and n of Incomplete Gamma-Hom model were estimated which maximize the log-likelihood function, lnL, (In most cases it is easy to work with the natural logarithm of the likelihood function, lnL, than L itself):

$$\ln L = -v_0 \ln\sigma - \frac{1}{2}\sum_{i=1}^{v_0}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 + \sum_{=v_0+1}^{v_1} \ln\Phi\left[\frac{(y_i)^c - \mu_i}{\sigma}\right] \qquad (3.13)$$

where

$$\Phi(Z) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{Z} e^{-\frac{z^2}{2}}\,dz \qquad (3.14)$$

where $$Z = \frac{(y_i^c - \mu_i)}{\sigma} \qquad (3.15)$$

Φ (z) is the standard normal cumulative distribution of z, v_0 number of uncensored data, v_1 total number of data including censored data, Y_i observed survival ratio, Y_i^c detection limit (which may differ for different trials), μ_i predicted survival ratio. When estimating parameters of the model the value for μ_i was replaced with the Incomplete Gamma-Hom model equation (Equation 3.9). Kinetic model parameter estimates were obtained solving equation 3.13 using solver routine of Microsoft® Excel 5.0 (Microsoft Canada, Mississauga, Ontario) to maximize lnL function.

3.2.8 Determination of marginal and joint confidence intervals

Marginal confidence intervals were calculated for model parameter estimates. Upper and lower 95% confidence limits were computed for each of the parameters using the likelihood ratio test (Seber and Wild 1989):

$$\ln L_{1-\alpha}(\beta) \geq \ln L_{max}(\hat{\beta}) - \frac{1}{2}\chi^2_{p,\alpha} \qquad (3.16)$$

where lnL(β) denotes the ln likelihood and $\ln L_{max}(\beta)$ its maximum value. Goal seek routine available in Microsoft® Excel 5.0 (Microsoft Canada, Mississauga, Ontario) was used to find marginal confidence limits by varying one parameter at a time with other parameters fixed at their optimum values. Joint confidence intervals were estimated for m and n in Incomplete Gamma Hom model with k fixed at its optimum value. Table function in Microsoft® Excel 5.0 (Microsoft Canada, Mississauga, Ontario) was used to compute joint confidence limits by varying two parameters at a time (m and n) with the other parameter (k) fixed to its optimal estimates such that the equality constraint was satisfied.

3.2.9 Validity of the models

For each model, regression residuals (observed - predicted inactivations) were plotted against all variables. Variables tested include; predicted and observed survival ratios, applied contact time, and initial chlorine dioxide residual.

3.3 RESULTS AND DISCUSSION

3.3.1 Results

Table 3.1 provides logistic dose response model parameter estimates for the four batches of oocysts collected during the course of the experimental program. Pearson chi-square statistics were calculated for Batch 20, 21, 22 and 23 and were found to be 3.2, 7.5, 2.2 and 5.8 respectively. The critical value of chi-square distribution for 95% significant limit are 9.5,9.5, 6.0 and 6.0 respectively. Hence there is no lack of fit between the observed proportions and logistic dose response model predicted proportions and the parameter estimates are considered to be valid.

Marginal confidence limits were constructed for each of the logistic dose response model parameter estimates using the likelihood ratio test and are provided in Table 3.1. The infectious dose needed to cause infection in cohort, ID_{50} was calculated for each batch of oocysts and are also provided in the Table 3.1.

Table 3.2, 3.3 and 3.4 provide the summary of chlorine dioxide information for the experimental trials at pH 6, 11 at 22°C and at pH 6 and 5°C respectively. Observed net inactivation ratios are also provided in each table.

Table 3.5 provides the maximum likelihood estimates of Incomplete Gamma Hom models for *C. parvum* oocysts inactivation by chlorine dioxide at pH 6, 11 at 22°C and at pH 6 and 5°C. Marginal confidence intervals and the model constraints are also provided. It is clear from these values that, three distinct set of model parameters exist which can adequately describe the inactivation of *C. parvum* oocysts at pH 6, 11 at 22°C and at pH 6 and 5°C.

3.3.2 Checks for model validity

Standard residual analysis was also carried out as outlined under the materials and methods. Results of residual analysis showed that, residuals are independent of all variables and randomly distributed with constant variances and approximately zero means. Figure 3.1, 3.2 and 3.3 show the fit of the observed inactivation data to the model predicted inactivation for chlorine dioxide at pH 6, 11 at 22°C and at pH 6 and 5°C respectively. Considering the inherent variation in animal dose response models, Figure 3.1, 3.2 and 3.3 show that proposed models can describe the observed data adequately within the wide range of inactivation observed. Analysis of these data showed that all three models do not violate the maximum likelihood assumptions.

3.3.3 Efficacy of chlorine dioxide

As mentioned in Chapter 1, only few studies have investigated the inactivation of *C. parvum* oocysts using chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). Out of those, only two studies (Korich et al. 1990b; Peeters et al. 1989) used animal infectivity as measures of post-disinfection oocyst viability. The other study (Ransome et al. 1993) used in vitro excystation.

Peeters et al. (1989) used low concentrations of chlorine dioxide (0.43 and 0.31 mg/L) for contact times up to 30 min. In their study, Peeters et al, (1989) observed about 1 log-unit inactivation of *C. parvum* oocysts by 0.43 mg/L chlorine dioxide applied for 30 min. In the present study the initial residual chlorine dioxide concentration of 0.5 mg/L applied for 30 min. resulted in 0.1 log-unit inactivation at pH 6. Therefore, it appears that the observations of the study of Peeters et al, (1989)

somewhat overestimate the inactivation of *C. parvum* by chlorine dioxide compared to the present study.

Data presented by Ransome et al. (1993) indicate that chlorine dioxide initial residual of 0.49 mg/L applied for 30 min. resulted in 0.4 log-units inactivation contrast to the 0.1 log-units predicted in the present study. Considering the possible variation in the results, it can be concluded that, the observations of the study of Ransome et al, (1993) are in agreement with the observations of the present study.

Korich et al. (1990a; 1990b) studied the effect of chlorine dioxide on *C. parvum* oocysts at pH 7 and 25°C . In vitro excystation was used as the main viability assay. However, animal infectivity using 3 to 6 day old neonatal BALB/c mice were also used to evaluate the viability for a few trials. Their inactivation data were not presented using the dose-response approach described in the present study. The ID_{50} that Korich et al. (1990a) reported for the BALB/c mice was about 60 oocysts, similar to that of Batch 21 (ID_{50} = 56) of the present study. Consequently, a crude estimation of inactivation can be made of Korich et al.(1990a) data using the dose-response model of the present study developed for CD-1 mice. This calculation gives 2 log-units inactivation of oocysts with 1.3 mg/L initial residual of chlorine dioxide for a contact time of 45 min. The analysis of the data reported by Korich et al, (1990a) is given in Table 3.5.

Assuming a first order chlorine dioxide decay rate of 0.005 $min.^{-1}$, predicted inactivation based on Incomplete Gamma-Hom model at pH 6, was compared to that of logistic dose response model estimated inactivation of Korich et al, (1990a) data . These results are also reported in Table 3.7. Results of the Table 3.7 show that, according to the Incomplete Gamma Hom model developed at pH 6 of the present study, 0.5 and 0.6

log-units of inactivation can be expected with 1.3 mg/L initial residual of chlorine dioxide for 45 and 60 min. of contact time respectively. However, logistic dose response analysis of mice responses reported by Korich et al, (1990a) results in 2.0 and 2.2 log-units of inactivation for the same level of chlorine dioxide application. Therefore it appears that the reported results of Korich et al. (1990a) overestimate the efficacy of chlorine dioxide when compared to the results of the present study. However, it should be noted that a number of significant assumptions were made in using the dose-response model developed in the present study for the reported data of Korich et al. (1990a).

Chlorine dioxide initial residual and contact time requirements for 1 log-unit inactivation of *C. parvum* oocysts are calculated based on the Incomplete Gamma-Hom models with a first order disinfection decay rate of 0.005 $min.^{-1}$ for pH 6 and 11, and are shown graphically in Figure 3.4. An immediate observation of the Figure 3.4 is the non-linearity of the models, and hence the invalidity of the CT (concentration X contact time) in describing the process design requirements for inactivation of *C. parvum* by chlorine dioxide. As a result, in the present study, comparisons of CT requirements for different degree of inactivation of *C. parvum* oocysts were avoided.

3.3.4 Effect of pH

Figure 3.4, clearly demonstrates that a higher inactivation of *C. parvum* oocysts is observed at higher pH for the same dose of chlorine dioxide. For example, for a contact time of 70 min., approximately 2.2 mg/L chlorine dioxide is required to achieve 1 log-unit inactivation at pH 6, however, for the same contact time, only about 0.9 mg/L of chlorine dioxide is required at pH 11.

A similar effect of pH was observed in inactivation of *Giardia* (Leahy 1985) virus (Alvarez and O'Brien 1982; Noss and Olivieri 1985) and *Naegleria gruberi* cysts (Sproul et al. 1983) by chlorine dioxide. Leahy (1985) reported CT requirements for 2 log-units inactivation of *G. muris* cysts at 25°C. He observed CT values of 4.8-6.8, 3.7-6.2 and 1.7-3.6 mg.min./L for pH 5, 7 and 9 respectively showing the higher efficacy of chlorine dioxide at higher pH. Alvarez and O'Brien (1982) observed approximately 1 log-units of inactivation of poliovirus with a 1 mg/L of chlorine dioxide for a contact time of about 2 min. at pH 6. However, with the same amount of chlorine dioxide they observed more than 3 log-units of inactivation of poliovirus at pH 10. A similar trend of effect of pH in inactivating *f2* virus was observed by pH 5, 7 and 9 at 3°C by Noss and Olivieri (1985). Sproul et al, (1983) observed a higher efficacy of chlorine dioxide at pH 9 compared to pH 5 and 7, at 25°C in inactivating *Naegleria gruberi* cysts.

These results are interesting because, as with ozone, even though to a lesser extent, chlorine dioxide decomposes fast at higher pH primarily to chlorite and to a certain extent, to chlorate and chloride. However, Noss and Olivieri (1985) observed in their study that, chlorite, chlorate and chloride had no inactivation effect on *f2* virus suggesting that, a chemical change in the virion particle at alkaline pH is responsible for higher inactivation of viruses at higher pH. Also, was shown in Chapter 2, that chlorite or chlorate has no effect in inactivation of *C. parvum* oocysts up to a concentration of 4 mg/L. Therefore, it appears (based on the observations of the present study) that, in case of *C. parvum* oocysts, the higher inactivation of oocysts observed with chlorine dioxide at higher pH is due to an effect of pH on the properties of the oocyst but not due to the effect of hydrolysis by-products (chlorite) of chlorine dioxide on oocysts.

3.3.5 Effect of temperature

To calculate the activation energy given by Arrhenius equation (equation 3.11), it is necessary to assume the same m and n parameters for I.G.H. model at 5° and 22°C. As can be seen in Table 3.5, m and n values were close, and mean values of m and n were selected for the Arrhenius model. Then the reaction rate constants were re-estimated and found to be 0.0169 and 0.0275 for 5° and 22°C respectively. Details of the Arrhenius models are provided in Table 3.6. Accuracy of the Arrhenius model predicted process design requirements were compared to that of process design requirements predicted by the I.G.H model and the results are given in Figure 3.5. Considering the experimental variation involved, it can be seen from Figure 3.5 that Arrhenius model predictions at 5° and 22°C are satisfactory.

The activation energy and Θ (equation 3.12) were calculated to be 4.7 kcal/mol and 1.029 1/min. respectively. This indicates that for every 10°C increase in temperature the reaction rate constant increases 1.3 folds. This is in contrast to the conventional approximate rule of thumb that the rate of reaction double for each 10°C rise in temperature. Gyürék et al, (1997) reported an activation energy of 3.8 kcal/mol for ozone inactivation of *C. parvum* at pH 7.0. Interestingly, Gyürék et al, (1997) also reported that for every 10°C increase in temperature the reaction rate constant increases 1.3 folds. These results indicates that ozone is a more effective oxidant compared to chlorine dioxide, but the effect of temperature on inactivation of *C. parvum* by ozone and chlorine dioxide is the same in magnitude.

An experiment was conducted at 13°C and pH 6 to cross validate the Arrhenius model at 13°C. Inactivation ratio of 4.5 log-units were observed with chlorine dioxide

initial residual concentration of 5.1 mg/L applied for 120 min. at 13°C at pH 6. The Arrhenius model at 13°C predicted inactivation of 4.9 log-units. Considering the inherent variation in experimental methodology these results are in reasonable agreement and the Arrhenius model prediction is considered satisfactory.

3.3.6 Process design requirements

Process design requirements for a 1 log-units of *C. parvum* oocysts inactivation at pH 6 and 11 are given in Figure 3.4. Process design requirements for a 1 log-units of *C. parvum* oocysts inactivation at 5°C and 22°C at pH 6 are given in Figure 3.5. These process design requirements were calculated based on the Incomplete Gamma-Hom model parameters provided in Table 3.5. A first order chlorine dioxide decay rate of 0.005 $min.^{-1}$ was assumed. Vertical axis provides the required amount of initial residual concentration of chlorine dioxide in mg/L for a given contact time (characteristic time of the contactor). The horizontal axis provides the required contact time in minutes. It should be noted that a safety factor was not included in calculating these process design requirements.

For example, from Figure 3.5, for a contact time of 90 min. 1.6 mg/L of initial residual chlorine dioxide should be applied to expect 1 log-units of inactivation of *C. parvum* oocysts at pH 6 and 22°C with 0.005 $min.^{-1}$ first order decay rate of chlorine dioxide. However, under the same conditions, for the same contact time, about 2.2 mg/L of initial residual concentration of chlorine dioxide is required to achieve the 1 log-units inactivation at 5°C.

The actual net inactivation of *C. parvum* oocysts by chlorine dioxide for a given chlorine dioxide contactor depends not only on the temperature, pH, chlorine dioxide concentration and contact time but also the hydraulic characteristics and the water quality. Therefore, for rational design, a relation between the distribution of residence times and the kinetics of the disinfection reaction is required (Trussell and Chao 1977). Since the Incomplete Gamma-Hom model takes the disinfectant decay in to account, the quality of the water may be characterized by the first order chlorine dioxide decay rate. Information of the disinfection kinetics for pH 6, 11 at 22°C and pH 6 at 5°C can be obtained from the Figures 3.4 and 3.5 respectively (for a first order chlorine dioxide decay rate of 0.005 $min.^{-1}$) or alternatively from Equation 3.9 substituting the corresponding model parameters given in Table 3.5 with a suitable first order chlorine dioxide decay rate. Therefore, once the hydraulic characteristics of the chlorine dioxide contactor has been established, information presented in this paper can be used to rationally design the chlorine dioxide contact chambers to achieve desired degree of inactivation of *C. parvum* oocysts at pH 6, 11 and 22°C and at pH 6 and 5°C.

3.4 CONCLUSIONS

Chlorine dioxide was found to be significantly more effective at pH 11 compared to pH 6 in inactivating *C. parvum* oocysts at 22°C. These findings support the reported data indicating the higher efficacy of chlorine dioxide in inactivating other amebic cysts and viruses at higher pH. Since it was observed in literature that, chlorine dioxide dissociation by-products has no cysticidal effect, the effect of pH is more likely due to changes in *C. parvum* oocysts which makes it more susceptible to chlorine dioxide at

higher pH. The data reported by Korich et al, (1990a; 1990b)and Peeters et al, (1989) on inactivation of *C. parvum* oocysts apparently overestimate the effect of chlorine dioxide compared to the results of the present study. However, it should be noted that number of critical assumptions were made in this comparison. Incomplete Gamma-Hom model, which account for the disinfectant decay, can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide.

Chlorine dioxide was found to be significantly more effective at 22°C compared to 5°C in inactivating *C. parvum* oocysts at pH 6. These findings support the literature reported data indicating the higher efficacy of chlorine dioxide in inactivating other amebic cysts and viruses at higher temperatures. Incomplete Gamma-Hom model, which accounts for the disinfectant decay, can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide at 22 and 5°C and at pH 6. Figures 3.4 and 3.5 provided can aid engineers in evaluating chlorine dioxide requirements for inactivation of *C. parvum* oocysts at 5° and 22°C and at pH 6.

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Table 3.1. Logistic dose response model parameters for different Batches of oocyst used

Oocyst batch number	Relevant trial numbers	$\hat{\beta}_0$	+/- 95% $\hat{\beta}_0$ limits	$\hat{\beta}_1$	+/- 95% $\hat{\beta}_1$ limits	ID_{50}	Natural logarithm of the Likelihood	Pearson chi-square statistic	No. of mice used to develop logit equation
20	754-810	-6.73	-7.6, -5.8	4.11	3.7, 4.6	43	-23.5	3.2	58
21	815-870	-6.80	-7.8, -5.9	3.89	3.5, 4.4	56	-27.4	7.5	59
22	919-934	-11.89	-12.7, -11.0	5.99	6.4, 5.6	96	-28.1	2.2	65
23	939-964	-4.53	-5.2, -3.8	2.42	2.8, 2.0	59	-37.5	5.8	74

Table 3.2. Summary of experimental settings for inactivation of *C. parvum* by chlorine dioxide at pH 6 and 22°C.

Experimental Trial	Applied dose, mg/L	Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Contact time, min.	First order chlorine dioxide decay rate, $min.^{-1}$	Observed net inactivation ratio, log-units	Incomplete Gamma Hom model predicted net inactivation ratio, log-units
760	2.0	2.1	1.4	120	0.004	1.8	1.8
768	2.0	2.2	1.3	90	0.006	1.5	1.5
770	4.0	4.5	4.0	30	0.004	1.9	1.9
772	0.5	0.5	0.2	118	0.007	0.2	0.2
774	4.0	4.4	3.0	90	0.005	3.8	3.8
776	0.5	0.5	0.4	30	0.010	0.0	0.1
863	4.0	4.5	2.4	179	0.004	>4.8	6.0

Table 3.3. Summary of experimental settings for inactivation of *C. parvum* by chlorine dioxide at pH 11 and 22°C.

Experimental Trial	Applied dose, mg/L	Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Contact time, min.	First order chlorine dioxide decay rate, $min.^{-1}$	Observed net inactivation ratio, log-units	Incomplete Gamma Hom model predicted net inactivation ratio, log-units
778	4.0	3.4	0.9	120	0.014	3.4	3.2
780	2.0	2.0	0.9	90	0.009	2.0	2.1
784	0.5	0.4	0.1	30	0.040	0.0	0.3
786	0.5	0.4	NM	120	0.040	0.0	0.3
793	2.0	2.1	0.5	120	0.012	2.5	2.3
795	4.0	3.8	2.1	90	0.007	3.6	3.6
797	4.0	4.0	3.2	30	0.008	1.8	1.9
867	3.5	3.6	0.9	180	0.007	4.7	4.8

Notes: NM not measured

Table 3.4. Summary of experimental settings for inactivation of *C. parvum* by chlorine dioxide at 5°C and at pH 6.

Experimental Trial	Applied dose, mg/L	Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Contact time, min.	First order chlorine dioxide decay rate, $min.^{-1}$	Observed net inactivation ratio, log-units	Incomplete Gamma Hom model predicted net inactivation ratio, log-units
919	1.4	1.5	1.1	120	0.0023	0.6	0.7
923	0.5	0.6	0.5	30	0.0040	0.2	0.1
943	4.5	4.8	4.2	60	0.0022	2.4	2.4
945	4.4	4.8	3.4	360	0.0010	>3.4	6.7
947	2.0	2.2	2.0	57	0.0017	1.0	0.8
949	2.0	2.2	1.3	360	0.0015	2.1	2.1
951	10.0	10.8	7.9	362	0.0009	>4.2	20.5
959	10.0	10.7	9.5	180	0.0007	>4.0	14.4

Table 3.5. Parameter estimates for Incomplete Gamma-Hom models for *C. parvum* oocysts inactivation by chlorine dioxide at pH 6 and 11 and at 22 °C.

Temp	pH	$\hat{k}$	+/- 95% $\hat{k}$ limits	$\hat{m}$	+/- 95% $\hat{m}$ limits	$\hat{n}$	+/- 95% $\hat{n}$ limits	$\hat{\sigma}$	Model Constraints: C= Conc. (mg/L) T = Time (min.)	Natural logarithm of the Likelihood	Number of trials
22	6	0.021	0.020 0.022	0.779	0.765 0.791	1.287	1.255 1.318	0.047	0.5≤C≤4.5 30≤T≤179	15.3	7
22	11	0.056	0.051 0.062	0.742	0.707 0.772	0.763	0.639 0.873	0.199	0.4≤C≤4.0 30≤T≤180	8.9	8
5	6	0.023	0.025 0.021	0.632	0.657 0.604	1.357	1.426 1.281	0.101	0.6≤C≤11.0 30≤T≤360	8.9	9

Table 3.6. Parameter estimates for Arrhenius Incomplete Gamma-Hom Models for *C. parvum* oocysts inactivation by chlorine dioxide at 5°, 13° and 22 °C and at pH 6.

Temp (°C)	$\hat{k}$	$\hat{m}$	$\hat{n}$	$\hat{\sigma}$	Model Constraints: C= Conc. (mg/L) T = Time (min.)	Natural logarithm of the Likelihood
22	0.027	0.706	1.322	0.085	0.6≤C≤4.5 and 30≤T≤179	11.8
13	0.021	0.706	1.322	0.085	0.6≤C≤4.5 and 30≤T≤179	
5	0.017	0.706	1.322	0.147	0.6≤C≤4.5 and 30≤T≤179	7.1

Table 3.7. Analysis of mice response data reported by Korich et. al., (1990a) at pH 7 and 25°C for chlorine dioxide using the logistic mice response model of the present study.

Initial chlorine dioxide residual (reported), mg/L	Contact time (reported), min.	Inoculum (reported), oocysts	Number of mice inoculated (reported)	Number of infected mice (reported)	Logit model calculated inactivation (assuming Batch 21), log-units	Incomplete Gamma Hom model (pH 6 and 22°C predicted net inactivation ratio, log-units
1.3	30	600	7	7	<0.6	0.4
1.3	45	600	9	2	2.0	0.5
		6000	11	6		
		60000	10	7		
1.3	60	600	8	0	2.2	0.6
		6000	10	8		
		60000	14	10		

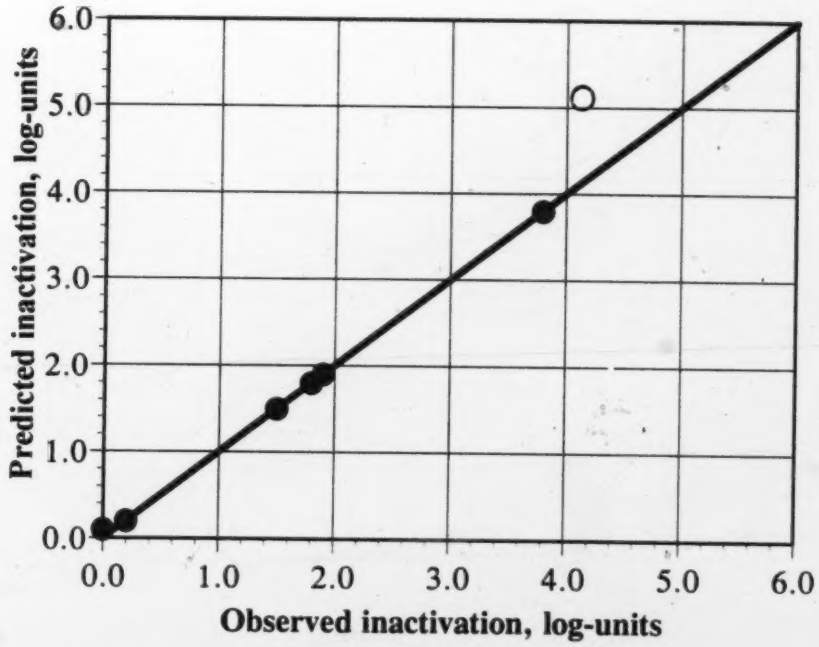

Figure. 3.1. Observed versus Incomplete Gamma Hom model predicted values for *C. parvum* oocysts inactivation by chlorine dioxide at pH 6.0 and 22°C (Solid line indicates the line of observed inactivation = to model predicted inactivation and open circles indicate the censored observations)

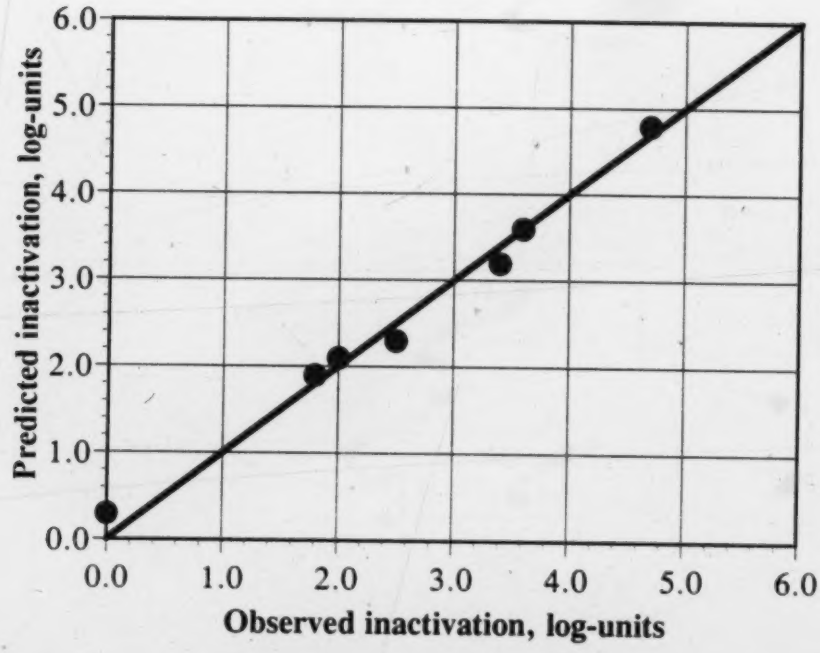

Figure. 3.2. Observed versus Incomplete Gamma Hom model predicted values for *C. parvum* oocysts inactivation by chlorine dioxide at pH 11.0 and 22°C (Solid line indicates the line of observed inactivation = to model predicted inactivation)

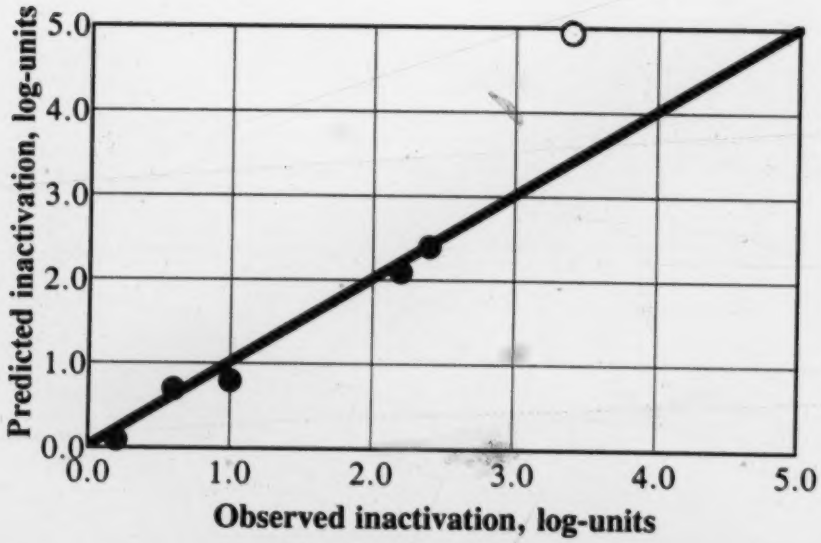

Figure. 3.3. Observed versus Incomplete Gamma Hom model predicted values for *C. parvum* oocysts inactivation by chlorine dioxide at pH 6.0 and 5°C (Solid line indicates the line of observed inactivation = to model predicted inactivation and open circles indicate the censored observations)

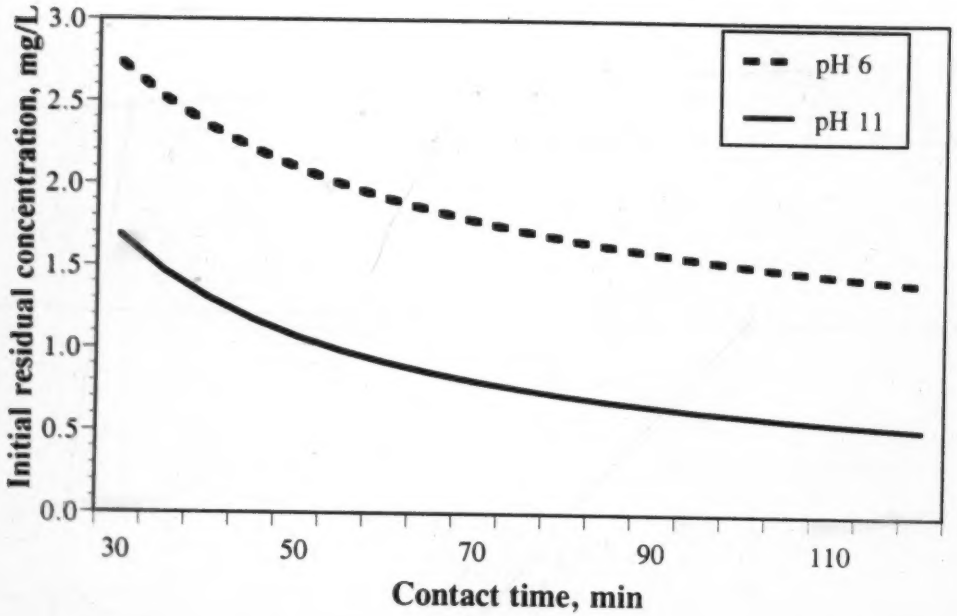

Figure. 3.4. Chlorine dioxide process requirements for 1 log-unit inactivation of *C. parvum* oocysts at pH 6 and 11 and 22°C for a first order chlorine dioxide decay rate of 0.005 min^{-1}.

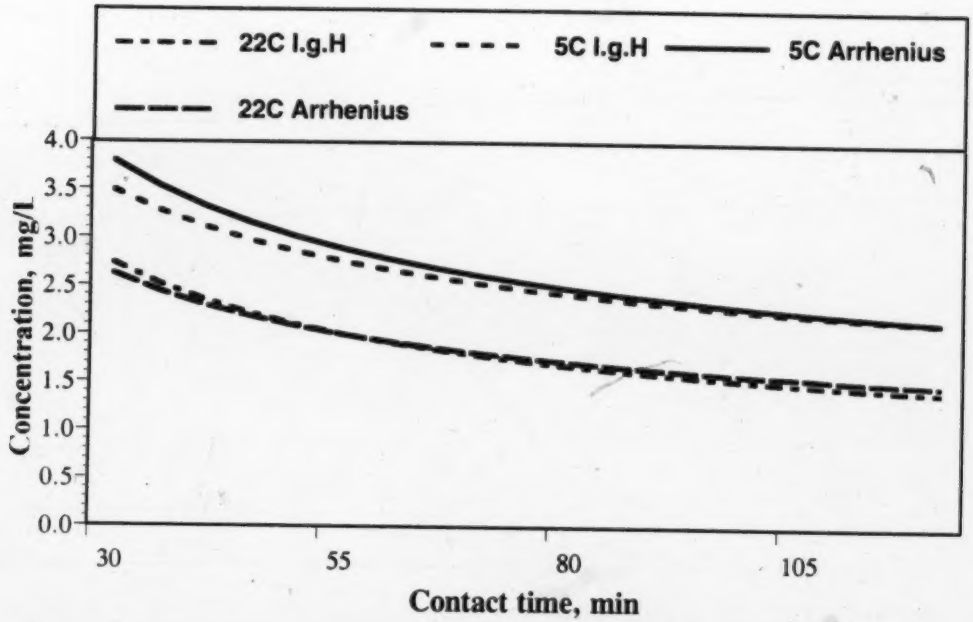

Figure. 3.5. Comparison of process requirements predicted by Incomplete Gamma Hom models and Arrhenius models for 1 log-unit inactivation of *C. parvum* oocysts at 5°C and 22°C at pH 6.0 for a first order chlorine dioxide decay rate of 0.005 min^{-1}.

Chapter 4

4. Synergism between chlorine dioxide and chlorine species in inactivating *C. parvum* oocysts[1]

4.1 INTRODUCTION

Environmental contamination of drinking water with the oocysts stage of *C. parvum*, excreted in the feces of infected human and animals can results in a diarrhoeal illness called cryptosporidiosis. The tough, nearly impervious wall of the oocyst has made disinfection by conventional methods ineffective (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987). Number of studies have proven that, ozone and chlorine dioxide are the most promising alternative disinfectants (Finch et al. 1993; Peeters et al. 1989).

Chlorine dioxide has a number of advantages over ozone including: the ability to retain a residual over a longer period of time; better selective reactivity; and low production of halogenated organics. Unfortunately, the maximum application level of chlorine dioxide is limited to about 1.4 mg/L, as applied chlorine dioxide, to meet currently regulated chlorine dioxide by-products level of 1.0 mg/L of total residual oxy-chlorine species. With this limitation, it may not be possible to achieve more than 1 log-unit (90%) inactivation by chlorine dioxide, when applied alone, under the usual contact times found in water treatment plant conditions (Chapter 3). Recent work has shown that, sequential disinfection with ozone followed by monochloramine, free chlorine

followed by monochloramine and ozone followed by chlorine dioxide were significantly more effective in inactivation of oocysts when compared with the disinfectants used singly (Finch et al. 1997b).

Number of other studies have been reported on the synergism between different disinfectants in inactivating bacteria and viruses. Kouame and Haas (1991) demonstrated that there is a synergistic interaction between free chlorine and monochloramine in killing *E.coli*. Katz et al., (1994) reported improved inactivation of fecal coliforms, fecal streptococci and *E. coliphages* when disinfected with chlorine dioxide and free chlorine. It was also reported that sodium hypochlorite and methanol combination was shown to have enhanced effect in inactivating *Bacillus subtilis* spores (Coates and Death 1978; Gorman et al. 1983). It was also shown that shaking of *C. parvum* oocysts with sand was to increase the sensitivity of oocysts to the secondary application of chlorine which resulted in increased inactivation (Parker and Smith 1993). Furthermore, number of research groups have demonstrated copper and/or silver when applied with chlorine to have a synergistic effect in inactivating bacteria and viruses (Abad et al. 1994; Shuval et al. 1995; Yahya et al. 1992). In another study, chlorine and bromine solutions were found to be effective in inactivating chlorine resistant bacteria (Farkas-Himsley 1964). Ozone and hydrogen peroxide (Hall and Sobsey 1993) and ultraviolet irradiation and hydrogen peroxide (Bayliss and Waites 1979) combinations were also shown to demonstrate synergism. However, no studies were found that

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[1] A version of this chapter is submitted for review in *Environmental Science and Technology*.

investigate the effect of sequential disinfection of *C. parvum* oocysts by chlorine dioxide and chlorine species.

The primary objective of this paper is to present the results of inactivation of *C. parvum* oocysts by sequential application of chlorine dioxide and chlorine species at pH 8 and 22°C. The secondary objective is to investigate the effect of pH and temperature on the synergism between chlorine dioxide and chlorine species.

4.2 MATERIALS AND METHODS

Procedures for chlorine dioxide generation, chlorine dioxide disinfection, animal infection, and subsequent calculation of inactivation are described in Section 2.2.1, 2.2.2, 3.2.1 and 3.2.2 respectively (also the details are provided in Appendices A and B).

4.2.1 Free chlorine and monochloramine

Free chlorine stock solutions were prepared daily as needed using sodium hypochlorite solution (6% available chlorine, BDH Inc., Poole, England) and oxidant demand-free water to give a concentration ranging from 300 to 600 mg/L. Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm), and phosphate buffer were made oxidant demand free following previously published procedures described in Appendix A. (Finch et al. 1994). Preformed monochloramine was prepared daily as needed by mixing equal volumes of free chlorine and ammonium chloride solutions (prepared from reagent grade ammonium chloride, AnalaR grade, BDH Inc., Poole, England), each prepared in 0.05M, phosphate buffer at a 3:1 (Cl_2:N weight) ratio yielding approximately 150 mg/L as chlorine solution. The DPD colorimetric procedure was used for determination of free and total chlorine

concentration. The fraction of the total chlorine measured as free chlorine in pre-formed monochloramine stock solutions was insignificant following 30 minutes of mixing. The stock chlorine and monochloramine solution was stored in dark refrigerated conditions during the day of the experiment.

4.2.2 Chlorine dioxide followed by free chlorine or monochloramine

At the end of the chlorine dioxide preconditioning contact time, residual chlorine dioxide was removed by purging with nitrogen gas for 15 min. at an approximate flow rate of 17 mL/min. After 15 minutes, the remaining chlorine dioxide concentration was less than 0.1 mg/L. Positive controls were used to account for any potential effect of residual chlorine dioxide concentration remaining and of nitrogen purging. Following nitrogen purging, the appropriate volume of free chlorine or monochloramine stock solution was added to the reactor. Initial and final free chlorine or monochloramine residuals were measured using the DPD colorimetric procedure. At the end of the contact time, residual chlorine or monochloramine was neutralized with 0.1 N sodium thiosulfate.

4.3 RESULTS AND DISCUSSION

Table 4.1 provides logistic dose response model parameters for different batches of oocysts used during the course of the experiments. Marginal confidence limits constructed using likelihood ratio test are also provided for each batch of oocysts. The infectious dose needed to cause infection in 50% of the population (ID_{50}) for different batches of oocysts range from 34 to 64 oocysts. Pearson chi-square statistics were calculated for Batch 17, 18, 19, 20 and 21 and were found to be 5.3, 2.0, 7.5, 3.2 and 7.5

respectively. The critical value of chi-square distribution for 95% significant limit are 16.9, 9.5, 11.1, 9.5 and 9.5 respectively. Hence there is no lack of fit between the observed proportions and logistic dose response model predicted proportions, and the parameter estimates are considered to be valid.

Marginal confidence limits were constructed for each of the logistic dose response model parameter estimates using the likelihood ratio test. Even though, the provided logistic dose response model parameter estimates for two logistic dose response models of Batch 17 and 18 were not significantly different, viability as measured by infectivity differed significantly amongst the other batches of oocysts, and hence these two batches were also regarded as separate. The infectious dose needed to cause infection in cohort, ID_{50} was calculated for all Batches and are provided in the Table 4.1.

Summary of experimental settings for sequential application of chlorine dioxide followed by free chlorine are provided in Table 4.2. As can be seen in Table 4.2, there are two levels of chlorine dioxide preconditioning followed by different levels of free chlorine treatment. The average high level of preconditioning with chlorine dioxide is equal to 1.4mg/L of initial residual concentration for 118 min. of contact time. The average low level of preconditioning with chlorine dioxide is equal to 1 mg/L of initial residual concentration for 45 min. of contact time. In the present study, it has been assumed that chlorine dioxide decomposition follows first-order kinetics and first-order decay rate constants are provided in Table 4.2. This appears to be a reasonable assumption based on our observations. However, this assumption remains to be verified over a wide range of water qualities. The first order decay rates were calculated using

measured residual chlorine dioxide concentrations (at least 6 observations) during the contact time of each experiment. The solver routine of Microsoft® Excel 5.0 (Microsoft Canada, Mississauga, Ontario), which uses the conjugate gradient method (Powell 1965), was used to minimize the error function to calculate the first order decay rate.

Summary of experimental settings for sequential application of chlorine dioxide followed by pre-formed monochloramine are provided in Table 4.3. As can be seen in Table 4.3, there are two levels of chlorine dioxide preconditioning followed by different levels of pre-formed monochloramine treatment. These preconditioning levels are virtually same to the preconditioning levels reported in Table 4.2 for chlorine dioxide followed by free chlorine trials.

Table 4.4 provides the observed inactivation ratios and expected inactivation for all trials including both chlorine dioxide followed by free chlorine as well as chlorine dioxide followed by monochloramine. Total expected inactivation is calculated based on the additive (log) expected inactivation of single compounds. The expected inactivation by chlorine dioxide at high level and low level of preconditioning was found experimentally. It was also found experimentally that no measurable inactivation can be expected at the levels of free chlorine treatments applied in the present study (8 mg/L initial residual concentration of free chlorine for 780 min. at pH 8). The results reported in the present study for free chlorine are consistent with those reported for *C. parvum* in earlier works (Finch et al. 1995; Korich et al. 1990a).

The inactivation due to monochloramine was calculated based on the Incomplete Gamma Hom model reported for inactivation of *C. parvum* oocysts by monochloramine at pH 8.0 and 22°C by Gyürék et al, (1997).

The Incomplete Gamma Hom model is given by (Haas and Joffe 1994):

$$\log\frac{N}{N_0} = \frac{-mkC_0^n}{(nk')^m}.\gamma(m,nk't) \qquad (4.1)$$

where m>0; nk't≥0; C_0 is the initial residual at time zero, mg/L; T is the contact time, min.; k' is the first order disinfectant decay rate constant $min.^{-1}$; k, n and m are kinetic parameters; and γ (m, nk't) is the Incomplete Gamma function. Reported values (Gyürék et al. 1997) for K, m and n parameters are $1.1x\,10^{-6}$, 1.28 and 2.53 respectively. Microsoft® Excel (Microsoft Canada, Mississauga, Ontario) was used to calculate the expected inactivation.

The additional inactivation observed in addition to the expected total inactivation is considered due to the synergism between chlorine dioxide and chlorine species. These results are also provided in Table 4.4.

4.3.1 Synergistic effect

Figure 4.1 shows the total observed inactivation and calculated inactivation due to synergism between chlorine dioxide and free chlorine with high band chlorine dioxide preconditioning (1.4 mg/L initial residual concentration for 120 min. contact time). Values in the horizontal axis show the corresponding CT value of free chlorine treatment. Figure 4.2 shows the total observed inactivation and calculated inactivation due to synergism between chlorine dioxide and free chlorine with low band chlorine dioxide preconditioning (1 mg/L initial residual concentration for 45 min. contact time). One of the immediate observation is that, synergism was observed between chlorine dioxide and free chlorine at all levels of free chlorine application. Another observation

is that the level of inactivation due to synergism increased with increasing CT of free chlorine for both high level of chlorine dioxide preconditioning (Figure 4.1) and for low level of chlorine dioxide preconditioning (Figure 4.2).

Another important observation is that, for a given level of free chlorine treatment, even though overall observed inactivation is high with high level of chlorine dioxide preconditioning, level of synergism observed is low compared to that with low level chlorine dioxide preconditioning. This observation clearly indicates that, there is an optimum level of chlorine dioxide preconditioning level at which maximum synergism can be observed between chlorine dioxide and free chlorine. The hypothesis behind the sequential disinfection is that the stronger oxidant conditions the outer membrane of the oocysts so that the secondary oxidant can penetrate the oocyst wall more readily. (There have been previous reports in the literature that support the above hypothesis (Finch et al. 1995)).

Therefore, there may exist a minimum or threshold level of preconditioning level to sufficiently sensitize the oocysts wall to notice any synergism between the preconditioner and secondary disinfectant. After the threshold level of preconditioning, synergism may increase with the level of preconditioning until the optimum preconditioning level. However, after the optimum preconditioning level, even though total inactivation is increased synergism may start to decline due to the excessive damage to the oocysts wall by the preconditioner inhibiting the action of secondary disinfectant.

Figure 4.3 shows the total observed inactivation and calculated inactivation due to synergism between chlorine dioxide and monochloramine with high band chlorine

dioxide preconditioning (1.4 mg/L initial residual concentration for 120 min. contact time). The horizontal axis provides the corresponding CT value of monochloramine treatment. As in the case of chlorine dioxide followed by free chlorine treatment, chlorine dioxide followed by monochloramine also showed synergism at all levels of preconditioning and monochloramine application. Another important observation is that, for a given level of monochloramine treatment, even though overall observed inactivation is high with high level of chlorine dioxide preconditioning, level of synergism observed is low compared to that with low level chlorine dioxide preconditioning. This further supports the observations made with chlorine dioxide and free chlorine combinations (Figures 4.1 and 4.2).

However, contrast to the results obtained with chlorine dioxide and free chlorine, the synergism between chlorine dioxide and monochloramine is not significantly increased with increase in monochloramine CT. This may be a result of difference in chemical nature of monochloramine compared to free chlorine.

Findings of the present study show similar results to the general pattern of synergism between stronger oxidant and a weaker oxidant when applied in sequence as noticed with ozone followed by chlorine or monochloramine, chlorine followed by monochloramine, and ozone followed by chlorine dioxide has been reported earlier (Finch et al. 1997b) with *C. parvum* oocysts.

4.3.2 Effect of pH and temperature on synergism between chlorine dioxide and chlorine species

Preliminary experiments were conducted to assess the effect of pH and temperature on synergism between chlorine dioxide and chlorine species. Experimental conditions are given in Table 4.5. These results are graphically shown in Figures 4.5 and 4.6.

Figure 4.5 shows the effect of pH (6, 8 and 11) on the synergism between chlorine dioxide and free chlorine and between chlorine dioxide and monochloramine. At pH 6 and 8 significant synergism was observed, whereas the observed synergism is low at pH 11 between both combinations. In case of chlorine dioxide followed by free chlorine combination, at pH 11, free chlorine completely exists as hypochlorite ions compared to about 77% hypochlorite ions and 23% hypochlorous acid at pH 8. Therefore it is possible that, even though hypochlorous acid can penetrate the preconditioned oocysts wall, hypochlorite ions may not be able to do so. It is known that oocysts are slightly negatively charged and since hypochlorite ions are also negatively charged, it is possible that they may repulse each other. However, it should be noted that pH not only affects the disinfectants but also the properties of oocysts. Therefore, the observed low synergism may, very well, be a combination of effect of pH on both disinfectants as well as the oocyst.

Chlorine dioxide and monochloramine also showed low synergism at pH 11, compared to pH 6 and 8. With the chlorine to ammonia weight ratio being 3: 1, both at pH 8 and 11, only monochloramine can be expected as the combined chlorine species present. Therefore, it is more likely that, the low synergism observed at pH 11 with

chlorine dioxide and monochloramine is as a result of effect of pH on oocysts rather than on chloramine distribution.

Figure 4.6 shows the effect of temperature on the synergism observed with chlorine dioxide and chlorine species. Under the same conditions applied, no synergism was observed at 5°C, compared to that of at 22°C at both pH 6 and 8. Except the fact that almost all chemical and biological reactions are slow at lower temperatures, temperature may also affect the properties of oocysts wall. However, these results are preliminary and further studies are highly recommended in this area. Further, a recent study showed that the synergism is also dependent upon the organism (Finch et al. 1997a). It was reported in that study that, synergism is more pronounced with difficult to kill protozoan cysts whereas, the synergism may not be observed with easy to kill organism such as, *Bacillus* spores.

From the above discussion it is clear that, synergism between chlorine dioxide and chlorine species strongly depend upon the level of preconditioning, level of secondary disinfectant application, pH, temperature, and the organism concerned. Synergism between chlorine dioxide and chlorine species is attractive, because it allows the use of low concentrations of each disinfectant to achieve a desired inactivation thereby reducing the disinfectant by-product risks. The sequential application of chlorine dioxide and chlorine species has other advantages as to the control of unwanted disinfectant by-products such as trihalomethane (Rav-Acha et al. 1985) in the absence of bromine. However, some other halogenated organics may be formed under these conditions. Formation of these halogenated organics may also be minimized using monochloramine instead of free chlorine in the absence of bromine. Therefore, it can be

inferred that in addition to the synergistic effect observed in the present study, sequential application of chlorine dioxide followed by free chlorine may results in low trihalomethane and halogenated organic byproduct formation. However, more studies are clearly needed to optimize the sequential application of chlorine dioxide and chlorine species with regard to all relevant variables.

4.4 CONCLUSIONS

The results of the present study provide new insight into the use of chlorine dioxide in conjunction with chlorine species. Specific findings were:

1. sequential disinfection with chlorine dioxide followed by free chlorine or monochloramine showed a significantly more inactivation of *C. parvum* oocysts due to the synergistic effect between chlorine dioxide and free chlorine or monochloramine at pH 6.0 and 8.0 at 22 °C.
2. there may exists an optimum level of preconditioning with chlorine dioxide above which there is no increase in synergism, even though the total inactivation is increased.
3. level of synergism is increased with the CT level of secondary disinfectant in case of free chlorine. Limited data showed that apparently synergism is not increased with respect to monochloramine CT level.
4. preliminary results show that, there is no synergism, at 5°C at pH 6 and 8, between chlorine dioxide and chlorine species investigated.
5. synergism between chlorine dioxide and chlorine species is a complex process and more studies are needed to understand the process more clearly.

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Table 4.1. Logistic dose response model parameters for different batches of oocyst used

Oocyst batch number	Relevant trial numbers	$\hat{\beta}_o$	+/- 95% $\hat{\beta}_o$ limits	$\hat{\beta}_1$	+/- 95% $\hat{\beta}_1$ limits	ID_{50}	Natural logarithm of the Likelihood	Pearson chi-square statistic	Critical value of D	No. of mice used to develop logit equation
17	595-635	-5.27	-5.9, -4.6	3.32	3.0, 3.7	34	-45.2	5.3	16.9	83
18	696-718	-5.64	-6.3, -4.9	3.17	2.8, 3.6	60	-34.9	2.0	9.5	56
19	724-745	-8.95	-9.7, -8.2	4.96	4.6, 5.4	64	-33.3	7.5	11.1	68
20	754-810	-6.73	-7.6, -5.8	4.11	3.7, 4.6	43	-23.5	3.2	9.5	58
21	815-870	-6.80	-7.8, -5.9	3.89	3.5, 4.4	56	-27.4	7.5	9.5	59

Table 4.2. Summary of experimental settings for inactivation of *C. parvum* by chlorine dioxide followed by free chlorine at pH 8 and 22°C.

Experimental Trial	Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Chlorine dioxide contact time, min.	First order chlorine dioxide decay rate, $min.^{-1}$	Initial free chlorine residual, mg/L	Final free chlorine residual, mg/L	Free chlorine contact time, min.	First order free chlorine decay rate, $min.^{-1}$
595	1.4	0.8	114	0.005	1.9	1.7	134	0.0018
628	1.4	0.7	120	0.006	0.0	0.0	120	0.0000
756	1.4	0.9	120	0.004	9.9	9.7	90	0.0002
758	1.5	0.7	120	0.006	7.6	4.0	364	0.0017
843	1.5	0.5	119	0.009	9.2	7.1	720	0.0004
845	1.5	0.9	119	0.004	1.9	1.8	720	0.0001
815	1.0	0.7	45	0.007	0.5	0.4	30	0.0038
817	1.0	0.7	45	0.010	0.5	0.3	360	0.0012
821	1.0	0.7	45	0.010	9.3	9.0	360	0.0001
839	1.0	0.8	45	0.006	9.8	9.1	30	0.0023
841	1.0	0.8	45	0.006	8.7	7.8	780	0.0001

Table 4.3. Summary of experimental settings for inactivation of *C. parvum* by chlorine dioxide followed by monochloramine at pH 8 and 22°C.

Experimental Trial	Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Chlorine dioxide contact time, min.	First order chlorine dioxide decay rate, $min.^{-1}$	Initial monochloramine residual, mg/L	Final monochloramine residual, mg/L	Monochloramine contact time, min.	First order monochloramine decay rate, $min.^{-1}$
724	1.5	0.9	119	0.004	0.6	0.6	30	0.0000
728	1.6	0.9	119	0.004	0.6	0.5	360	0.0001
732	1.5	0.6	119	0.007	5.9	5.7	360	0.0001
738	1.5	0.5	119	0.009	11.7	11.4	120	0.0002
742	1.5	0.6	120	0.007	2.8	2.6	180	0.0003
744	1.5	0.7	132	0.006	11.8	11.4	363	0.0001
825	1.0	0.7	45	0.009	0.6	0.5	360	0.0002
827	1.0	0.7	45	0.007	0.6	0.6	30	0.0000
829	1.0	0.7	45	0.007	9.2	9.0	30	0.0008
833	1.0	0.8	45	0.006	9.0	8.8	360	0.0001
835	1.0	0.7	45	0.009	9.3	8.6	720	0.0001

Table 4.4. Observed inactivation and estimated inactivation due to synergism for all the trials

Preconditioning band and secondary disinfectant	Trial number	Net inactivation ratio, log-units	Inactivation ratio due to synergism, log-units
High preconditioning, 1.4 mg/L chlorine dioxide for 120 min., chlorine	595	2.0	0.3
	628	1.7	0.0
	756	2.3	0.6
	758	2.9	1.3
	843	3.7	2.1
	845	2.4	0.7
Low preconditioning, 1 mg/L chlorine dioxide for 45 min., chlorine.	815	1.1	0.6
	817	1.6	1.1
	821	2.1	1.6
	823	1.7	1.2
	841	2.7	2.2
High preconditioning, 1.4 mg/L chlorine dioxide for 120 min., monochloramine.	724	2.2	0.5
	728	2.6	0.9
	732	2.9	1.0
	738	2.8	0.9
	742	2.5	0.8
	744	3.1	0.5
Low preconditioning, 1 mg/L chlorine dioxide for 45 min., monochloramine.	825	1.8	1.3
	827	1.5	1.0
	829	2.3	1.8
	833	2.0	1.0
	835	2.8	1.3

Table 4.5. Summary of additional trials conducted to investigate the effect of pH and temperature.

Trial no:	Temp, °C	pH	Chlorine dioxide initial residual, mg/L	Chlorine dioxide contact time, min.	Secondary disinfectant	Secondary disinfectant initial residual, mg/L	Secondary disinfectant contact time, min.	Total inactivation observed, log-units	Inactivation due to synergism, log-units
1	22	6	1.2	119	Free chlorine	1.8	120	2.2	1.2
2	22	8	1.3	120	Free chlorine	1.6	120	3.0	1.7
3	22	11	1.3	119	Free chlorine	2.1	120	2.2	0.6
4	22	6	1.2	120	Monochloramine	2.1	120	2.2	1.2
5	22	8	1.5	120	Monochloramine	2.8	180	2.8	1.3
6	22	11	1.3	120	Monochloramine	2.0	120	2.1	0.5
7	5	6	2.0	120	Free chlorine	1.7	120	0.8	0.0
8	5	8	2.0	120	Free chlorine	1.5	120	0.8	0.0
9	5	6	1.6	119	Monochloramine	2.1	120	0.6	0.0
10	5	8	1.5	120	Monochloramine	2.5	120	0.6	0.0

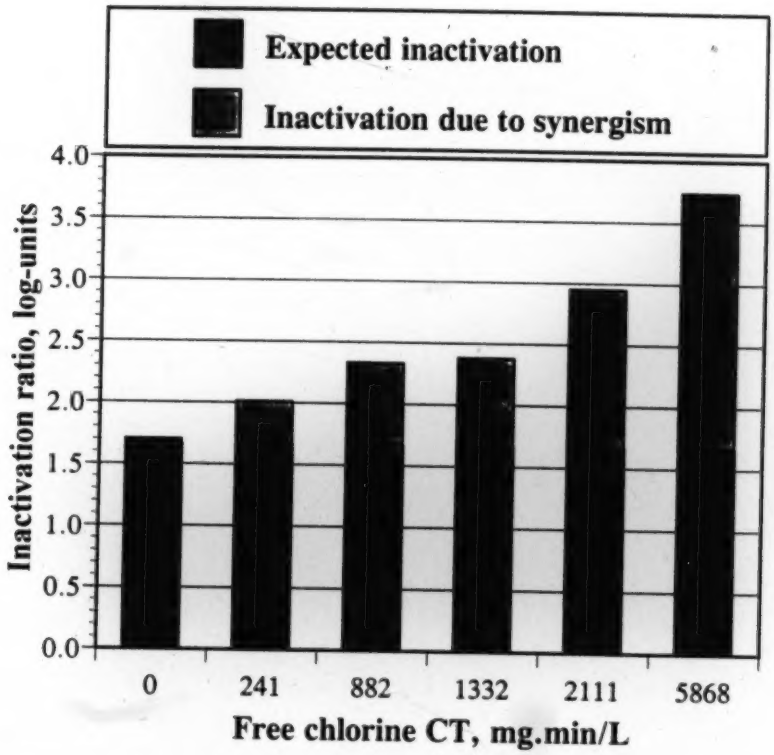

Figure 4.1. Expected inactivation and inactivation due to synergism between chlorine dioxide and free chlorine at pH 8 and 22°C. Chlorine dioxide high level preconditioning with 1.4 mg/L for 120 min.

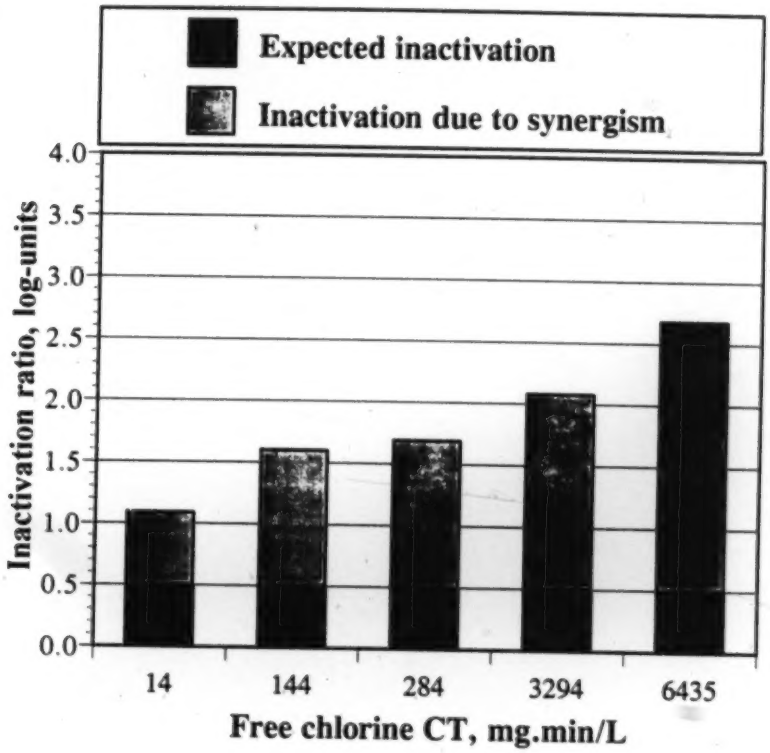

Figure 4.2. Expected inactivation and inactivation due to synergism between chlorine dioxide and free chlorine at pH 8 and 22°C. Chlorine dioxide low level preconditioning with 1 mg/L for 45 min.

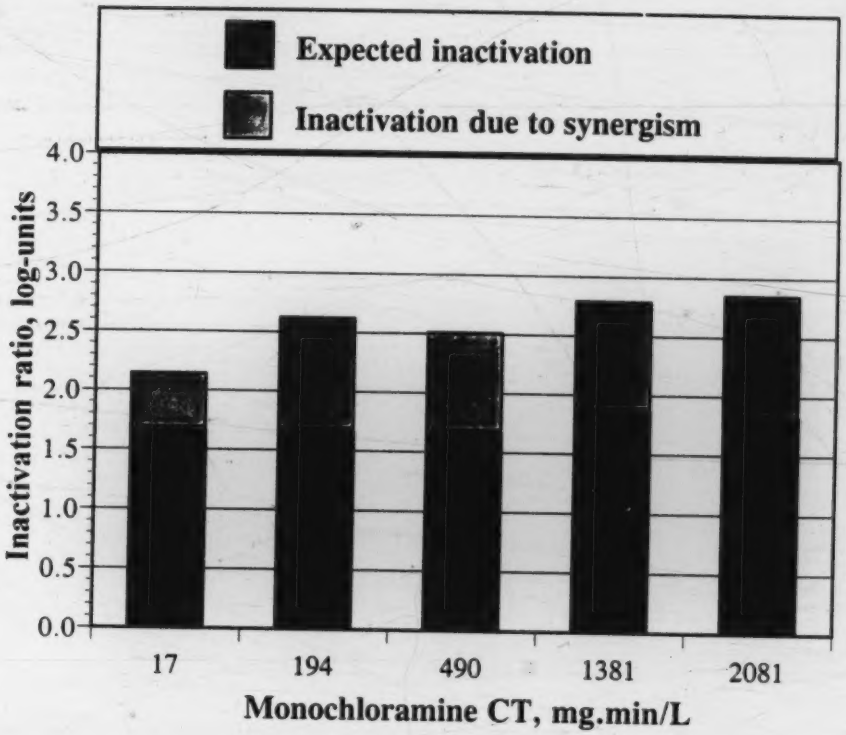

Figure 4.3. Expected inactivation and inactivation due to synergism between chlorine dioxide and monochloramine at pH 8 and 22°C. Chlorine dioxide high level preconditioning with 1.4 mg/L for 120 min.

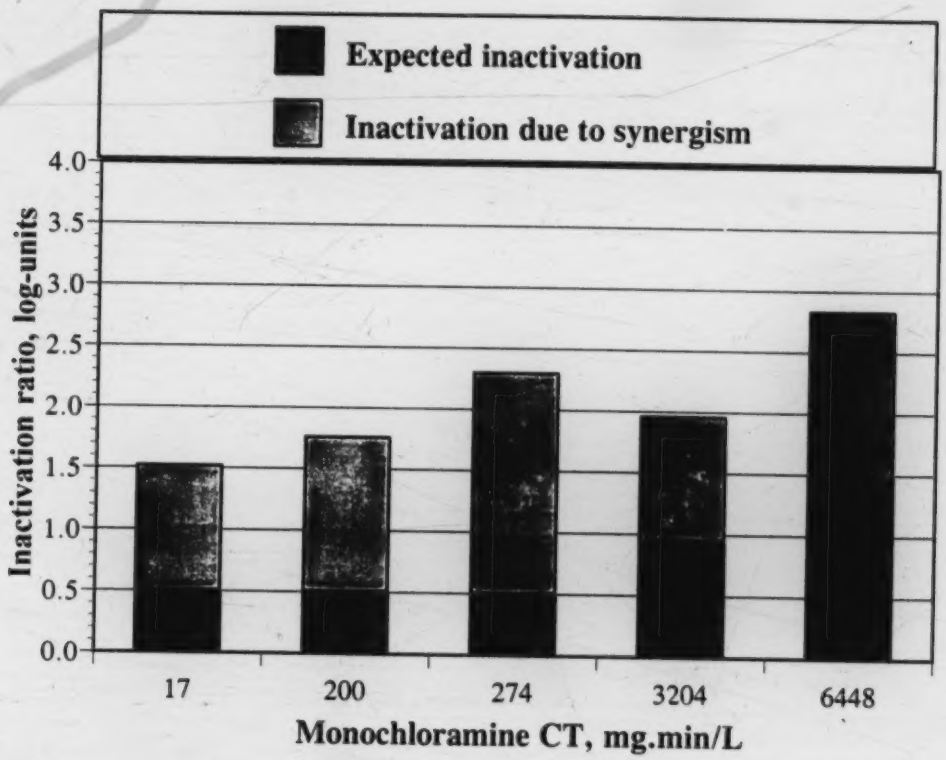

Figure 4.4. Expected inactivation and inactivation due to synergism between chlorine dioxide and monochloramine at pH 8 and 22°C. Chlorine dioxide low level preconditioning with 1 mg/L for 45 min.

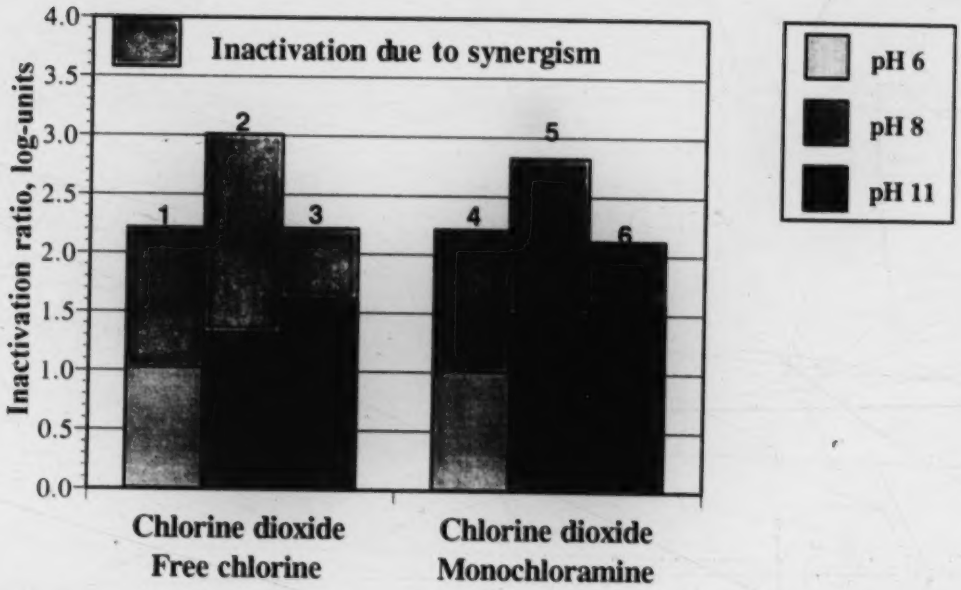

Figure 4.5. Effect of pH on synergism between chlorine dioxide and chlorine species at 22°C. For experimental conditions refer the numbers above the columns to the data provided in Table 4.5.

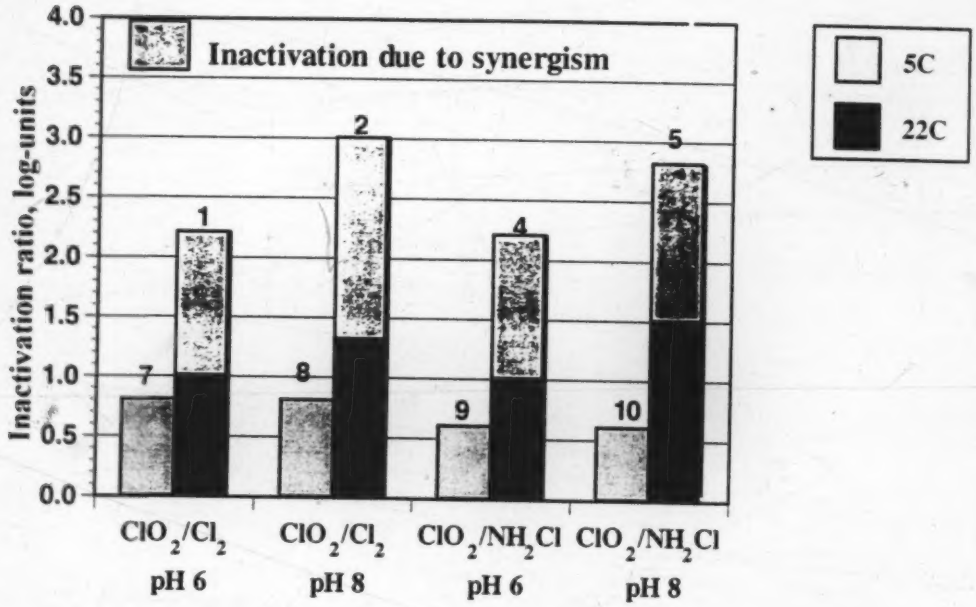

Figure 4.6. Effect of temperature on synergism between chlorine dioxide and chlorine species at pH 6 and 8. For experimental conditions refer the numbers above the columns to the data provided in Table 4.5.

Chapter 5

5. Sequential disinfection of *C. parvum* by ozone and chlorine dioxide[1]

5.1 INTRODUCTION

Waterborne cryptosporidiosis outbreaks have been documented in Canada, the United States and Great Britain (Gallaher et al. 1989; Hayes et al. 1989; MacKenzie et al. 1994; Pett et al. 1994; Richardson et al. 1991). Many studies have shown that chlorine, monochloramine and ultraviolet irradiation at practical plant doses are ineffective in controlling *C. parvum* oocysts (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993). Among the alternative disinfectants, ozone and chlorine dioxide appear to be the most promising (Finch et al. 1993; Peeters et al. 1989). Chlorine dioxide has number of advantages when compared with ozone including: the ability to retain a residual over a longer period of time; better selective reactivity; and low production of halogenated organics (Miller et al. 1978; Rav-Acha et al. 1985).

Ozone is the most effective single disinfectant against *C. parvum* oocysts (Korich et al. 1990b; Peeters et al. 1989). However, one of the disadvantages of ozone is that it is unstable in water making it difficult to maintain disinfectant residuals in the distribution systems. As a result, ozone is commonly used as the primary disinfectant with a secondary disinfectant used for distribution system protection.

Chlorine dioxide is limited to about 1.2 to 1.4 mg/L as applied chlorine dioxide to meet the currently regulated chlorine dioxide by-products level of 1.0 mg/L of total

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[1] A version of this chapter is published in Ozone Science and Engineering Vol. 19 , pp. 409-423

residual oxy-chlorine species (Werdehoff and Singer 1987). With this limitation, it may not be possible to achieve more than 1 log-unit inactivation by chlorine dioxide under the usual contact times found in water treatment plants (Chapter 3).

Sequential disinfection with ozone followed by monochloramine or free chlorine followed by monochloramine were significantly more effective in inactivation of oocysts when compared with the disinfectants used singly (Finch et al. 1995). However, no literature was found which investigated the inactivation of *C. parvum* oocysts by sequential disinfection with ozone followed by chlorine dioxide.

The objective of this paper is to present the results of inactivation of *C. parvum* oocysts by sequential application of ozone and chlorine dioxide in which ozone was applied first followed by chlorine dioxide.

5.2 MATERIALS AND METHODS

Procedures for chlorine dioxide generation, chlorine dioxide disinfection, animal infection and subsequent calculation of inactivation are described in Section 2.2.1, 2.2.2, 3.2.1 and 3.2.2 respectively. Details are also provided in Appendices A and B.

5.2.1 Ozone generation

Ozone gas was generated using a water-cooled, corona discharge generator (Model T-816, Welsbach Ozone Systems Corporation, Sunnyvale, CA) from extra dry oxygen feed gas. Oxygen carrier gas containing approximately 5 per cent ozone was bubbled for a minimum of 20 min. at 22°C through 400 mL of Elga® water in a 500 mL gas absorption flask. Ozone concentration in the stock solution was 20±1 mg/L. Ozone residuals were determined by ultraviolet absorbance at 260 nm using a molar

absorbtivity of 3,300 $M^{-1} \cdot cm^{-1}$ (Hart et al. 1983). The ozone solution was used within 3 min. of removing it from the gas stream.

5.2.2 Ozone followed by chlorine dioxide

The test preparation of 1.5×10^7 oocysts was suspended in 400 mL of oxidant demand-free, 0.05 M phosphate buffer at pH 8. The reactor vessel was a 500 mL borosilicate Wheaton Media/Lab bottle with a Teflon®-coated magnetic stir bar for agitation using a magnetic stirrer (Thermix® Model 220T, Fisher Scientific Ltd., Pittsburgh, PA). The concentration of the stock ozone solution was measured four times prior to and immediately following addition of a calculated volume of ozone solution to the reactor with a calibrated pipette. The reactor was continuously sampled for residual concentration as described above.

Residual ozone was neutralized with 1.0 M sodium formate prepared from reagent grade sodium formate (AnalaR grade, BDH Inc., Poole, England). The mass of sodium formate added did not have an interfering effect at absorbance at 260 nm and has been found to have no effect on the oocysts as shown by positive controls. Following addition of 1.0 M sodium formate, a measured volume of chlorine dioxide from a concentrated stock solution was added to the reactor and stirred. The concentration of chlorine dioxide in aqueous solution was measured as described above. At the end of the contact time, the residual chlorine dioxide was removed as described above.

5.3 DATA ANALYSIS

5.3.1 Kinetics of disinfection

Integral Hom model developed by Finch et al.(1997) (derivation of equation 6.1 is given in Chapter 3) was used to describe the *C. parvum* oocyst inactivation by chlorine dioxide with and without ozone pretreatment. This model account for the decreasing disinfectant residuals:

$$\log\left(\frac{N}{N_0}\right) = -kmC_0^n \int_0^T e^{-k'm} t^{m-1} dt \qquad (5.1)$$

where t is the elapsed time in the interval from zero to T.

5.4 RESULTS AND DISCUSSION

5.4.1 Chlorine dioxide alone

The experimental conditions used for inactivation of *C. parvum* oocysts by chlorine dioxide alone are summarized in Table 5.1. Initial residual concentration of chlorine dioxide ranged from 0.40 to 3.3 mg/L and contact times from 15 to 120 minutes. The first-order decay rates were calculated using measured residual chlorine dioxide concentrations (at least 6 observations) over the duration of the contact time. The mean first-order chlorine dioxide decay rate was 0.035 $min.^{-1}$ with upper and lower 95 per cent confidence limits of 0.042 and 0.028 $min.^{-1}$, respectively.

There have been few studies reported on the inactivation of *C. parvum* oocysts using chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). Only two studies used animal infectivity as the measure of post-disinfection oocyst

viability (Korich et al. 1990b; Peeters et al. 1989). The other study (Ransome et al. 1993) used in vitro excystation. When different methods are used to estimate the infectiousness of disinfected oocysts the results are difficult to compare since there is evidence that excystation underestimates the degree of inactivation (Black et al. 1996; Owens et al. 1994).

Peeters et al. (1989) used low concentrations of chlorine dioxide (0.43 and 0.31 mg/L) for contact times of up to 30 min. They observed about 1 log-unit inactivation of *C. parvum* oocysts after 30 min. when using an applied dose of 0.43 mg/L chlorine dioxide.

Ransome et al. (1993) found that when chlorine dioxide had an initial residual of 0.49 mg/L, there was 0.4 log-units inactivation after 30 min. as measured by in vitro excystation. In the present study, an initial residual chlorine dioxide concentration of 0.36 mg/L when applied for 58 min. resulted in 0.6 log-units of inactivation. Given the degree of variability in the animal model for low inactivation and the different protocols used in the studies including pH and temperature conditions, these results are in reasonable agreement with one another.

Korich et al. (1990a; 1990b) studied the effect of chlorine dioxide on *C. parvum* oocysts at pH 7 and 25°C. In vitro excystation was used as the main viability assay. However, animal infectivity using 3 to 6 day old neonatal BALB/c mice were also used to evaluate the loss of infectiousness for a few trials. Their inactivation data were not presented using the dose-response approach described by Finch et al. (1994). The ID_{50} that Korich et al. (1990b) reported for the BALB/c mice was about 60 oocysts, similar to that of Finch et al. (1994). Consequently, a crude estimation of inactivation can be

made of Korich et al. (1990b) data using the dose-response model of Finch et al. (1994) developed for CD-1 mice. When this calculation was performed, it was found that 2 log-units of inactivation of oocysts was expected after 60 min. when the initial chlorine dioxide residual was 1.3 mg/L. The present study found that 1.6 log-units inactivation was achieved with 1.6 mg/L of initial chlorine dioxide residual after 120 min.

5.4.2 Ozone and chlorine dioxide sequentially

The experimental conditions used for inactivation of *C. parvum* oocysts by ozone followed by chlorine dioxide are tabulated in Table 5.2. Ozone doses ranged from 0.7 to 0.8 mg/L initial residual and contact times of 5.4 to 4.3 min. followed by chlorine dioxide initial residuals of 0.36 to 2.0 mg/L with contact times ranging from 60 to 360 min.

An immediate observation from Table 5.2 is that there is a marked difference between the first-order decay rates of ozone and chlorine dioxide in the test water. The mean first-order ozone decay rate was 0.18 $min.^{-1}$ with upper and lower 95 per cent confidence limits of 0.23 and 0.12 $min.^{-1}$, respectively. The mean first-order chlorine dioxide decay rate after pre-ozonation was 0.0056 $min.^{-1}$ with upper and lower 95 per cent confidence limits of 0.013 and -0.0015 $min.^{-1}$, respectively. Note that the confidence limits include zero indicating that the chlorine dioxide decay rate was indistinguishable from zero.

When comparing the chlorine dioxide decay rates in Table 5.1 with those in Table 5.2, it is apparent that ozone is probably oxidizing material that caused chlorine dioxide demand in the previous experiments.

The first trial of the Table 5.2 gives inactivation of *C. parvum* by ozone, when applied alone. Peeters et al. (1989) reported 2 log-unit inactivation of *C parvum* oocysts when exposed to 1.06 mg/L of ozone for a contact time of 5 min. In the present study, 0.8 log-units inactivation of oocysts was observed when exposed to 0.9 mg/L of ozone for 3.5 min. at pH 8.

Another important observation is that there appears to be a synergistic effect between ozone and chlorine dioxide. Figure 5.1 illustrates the synergism of using ozone and chlorine dioxide sequentially. There was a 3.4 log-unit reduction in infectivity when oocysts were exposed to an ozone pretreatment of 0.8 mg/L for 4.4 min. followed by a chlorine dioxide residual of 2.0 mg/L for 60 min. The expected inactivation by single oxidants was 0.8 and 1.4 log-units for a total of 2.2 log-units, due to ozone and chlorine dioxide, respectively. The sequential treatment of oocysts by ozone and chlorine dioxide resulted in an additional 1.2 log-units of inactivation due to the synergism of the two disinfectants. These preliminary findings indicate that sequential disinfection with ozone followed by chlorine dioxide may have potential in controlling waterborne parasites.

This finding is significant given the expectations of more stringent regulation of chlorine dioxide species in drinking water. With the expected more stringent regulation of chlorine dioxide species in drinking water chlorine dioxide alone, may not result in sufficient inactivation of *C. parvum* oocysts under general water treatment plant conditions. The results of the present study suggest that sequential application of ozone followed by chlorine dioxide may be used to overcome these limitations.

In addition to the significant synergistic effect observed with ozone and chlorine dioxide, there are other advantages. Ozone residuals under water treatment plant conditions are not stable thereby making it impractical to produce residual disinfection with ozone alone. On the other hand, even though chlorine dioxide is a weaker oxidant compared to ozone, chlorine dioxide is very stable and may provide sufficiently long residual disinfection. Another potential advantage is that chlorine dioxide is selective in its reactions and produces no trihalomethanes as a disinfection byproduct.

Another practical outcome of this potent combination is that disinfection processes can be designed to use less ozone and less chlorine dioxide to achieve the degree of kill desired when compared to using the disinfectants singly. Therefore application of ozone and chlorine dioxide in sequence may have the potential of providing the advantages of both oxidants under appropriate conditions.

5.4.3 Development of design criteria

A suitable kinetic model has not been reported for inactivation of *C. parvum* oocysts by chlorine dioxide. The data reported in Table 5.1 and 5.2 were used to estimate the parameters for the Integral-Hom model (Equation 5.1). The maximum likelihood parameter estimates are summarized in Table 5.3.

Figure 5.2 shows that the fit of the observed inactivation data to the model predicted inactivation for chlorine dioxide without ozone preconditioning is reasonable. Figure 5.3 shows that the fit of the observed inactivation data to the predicted inactivation for sequential application of ozone followed by chlorine dioxide is also reasonable.

Process design requirements for *C. parvum* oocysts inactivation by chlorine dioxide with and without ozone pretreatment are compared in Figure 5.4.

Figure 5.4 shows that, under the currently regulated level of chlorine dioxide application (1.0 mg/L of chlorine dioxide, chlorite and chlorate residual), 3 log-unit inactivation of *C. parvum* oocysts may be achieved under general water treatment plant conditions with ozone pretreatment of 0.7 mg/L of initial ozone residual for a contact time of 5 min. For example, a chlorine dioxide contactor having a contact time of 75 min., requires an initial residual of 2.7 mg/L of chlorine dioxide for 3 log-units of inactivation. Application of this level of chlorine dioxide may not be practical. However, for the same chlorine dioxide contactor, an initial residual of only 1.0 mg/L is required to achieve the same level of inactivation when the water is pretreated with 0.7 mg/L of initial residual ozone for 5 min.

As shown in Table 5.2, ozone pretreatment resulted in average first order chlorine dioxide decay rate of 0.006 $min.^{-1}$. Therefore, part of the increased efficiency of chlorine dioxide observed when pretreated with ozone can be attributed to the lower decay of chlorine dioxide resulting from the ozone pretreatment. The chlorine dioxide requirements for a first order decay rate of 0.006 min^{-1} (without ozone pretreatment) also are shown in Figure 5.4. This shows that the significant amount of reduced chlorine dioxide requirements when pretreated with ozone can be attributed to the lower rate of chlorine dioxide decay resulting from ozone pretreatment.

However, it should be noted that the kinetic model developed for sequential application of ozone followed by chlorine dioxide was developed with a limited number

of data. More data are needed to better understand the behavior and threshold limits, if any, of the primary and secondary disinfectants.

5.5 CONCLUSIONS

The sequential treatment of *C parvum* oocysts by ozone followed by chlorine dioxide resulted in additional inactivation due to the synergism of the two disinfectants. With ozone pretreatment, chlorine dioxide may effectively be used to achieve inactivation of *C. parvum* oocysts up to 3 log-units while meeting current U.S. Environmental Protection Agency regulatory limits for chlorine dioxide application. This combination of disinfectants may have potential in controlling waterborne parasites and hence is a candidate for further investigation.

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Table 5.1. Summary of data used to estimate the parameters for inactivation of *C. parvum* by chlorine dioxide at pH 8 and 22°C.

Initial chlorine dioxide residual, mg/L	Final chlorine dioxide residual, mg/L	Contact time, min.	First-order decay rate constant, $min.^{-1}$	Observed inactivation ratio, log-units	Predicted inactivation ratio, log-units
0.4	0.07	58	0.029	0.0	0.6
1.0	0.06	58	0.047	0.5	0.8
1.0	0.42	29	0.030	0.0	0.8
1.4	0.58	30	0.030	1.0	1.0
1.5	0.04	61	0.060	1.6	1.0
1.6	0.02	120	0.037	1.6	1.6
1.8	0.33	59	0.029	2.5	1.7
1.9	0.07	120	0.028	1.8	2.4
2.0	1.04	15	0.044	0.5	0.7
2.7	0.02	120	0.041	3.2	2.2
2.9	0.65	59	0.025	2.0	2.6
3.3	0.37	120	0.019	>3.2	4.4

Table 5.2. Summary of data used to estimate the parameters for inactivation of *C. parvum* by ozone followed by chlorine dioxide at pH 8 and 22°C.

Trial number	Initial residual, A*, mg/L	Final residual, A, mg/L	First order decay rate, A, $min.^{-1}$	Contact time, A, min	Initial residual, B**, mg/L	Final residual, B, mg/L	First order decay rate, B, $min.^{-1}$	Contact time, B, min.	Observed inactivation ratio, log-units	Predicted inactivation ratio, log-units
715	0.76	0.4	0.17	4.5	0.4	0.2	0.0068	60	2.0	1.9
707	0.82	0.4	0.17	4.3	0.4	0.2	0.0016	360	3.4	3.3
630	0.87	0.5	0.14	4.0	1.1	0.8	0.0040	60	2.5	2.8
717	0.69	0.2	0.24	5.4	1.4	1.1	0.0007	360	>4.8	5.4
705	0.80	0.5	0.11	4.4	2.0	1.8	0.0150	60	3.6	3.4

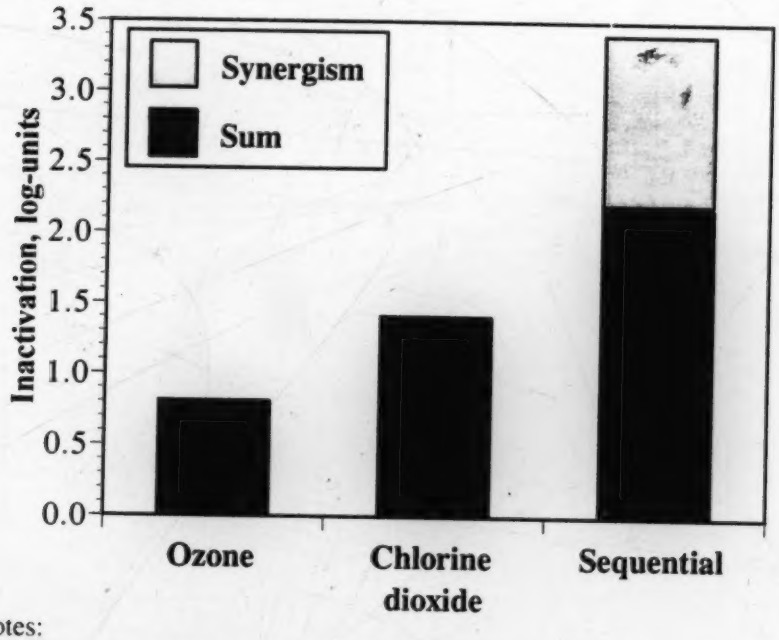
* Ozone

** Chlorine dioxide

Table 5.3. Parameter estimates for Integral-Hom models for *C. parvum* oocysts inactivation by chlorine dioxide with and without ozone pretreatment at pH 8.0 and temperature 22°C.

Condition	$\hat{k}$	$\hat{m}$	$\hat{n}$	$\hat{\sigma}$	Number of trials
Without ozone pretreatment	0.043	0.93	0.70	0.56	12
With ozone pretreatment	0.80	0.31	0.38	0.22	5

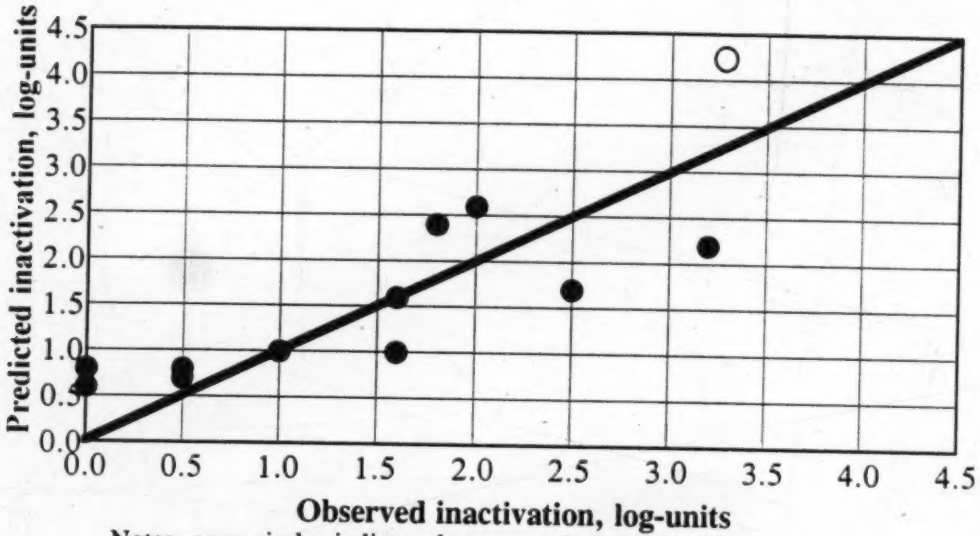
Note: 0.7 to 0.8 mg/L ozone initial residual for contact time of 4.0 to 5.4 min.



Notes:

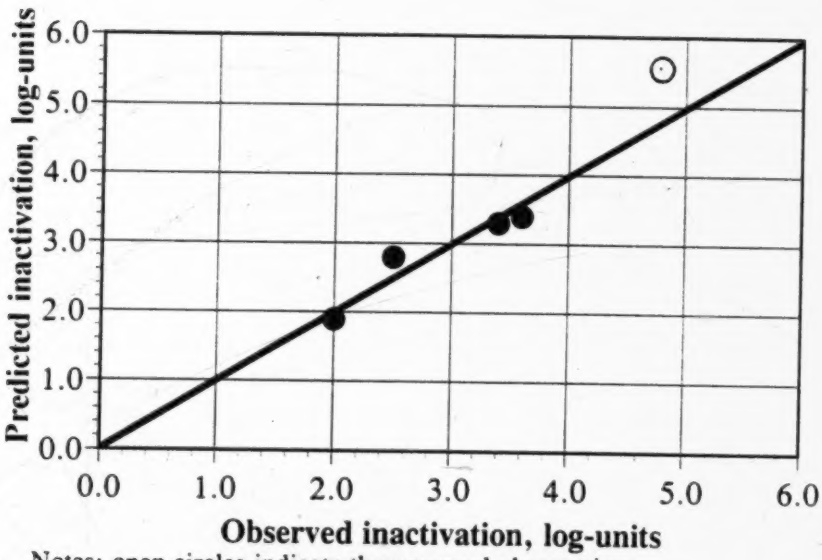
1. Ozone: 0.9 mg/L of initial residual for contact time of 3.5 min
2. Chlorine dioxide: 2.0 mg/L of initial residual for contact time of 60 min
3. Ozone: 0.8 mg/L of initial residual for contact time of 4.4 min followed by chlorine dioxide 1.95 mg/L of initial residual for contact time of 60 min

Figure 5.1. Synergistic effects of sequential disinfection of *C. parvum* by ozone followed by chlorine dioxide at pH 8.0 and 22°C.



Notes: open circles indicate the censored observations

Figure 5.2. Observed versus model predicted values for *C. parvum* oocysts inactivation by chlorine dioxide without ozone pretreatment at pH 8.0 and 22°C



Notes: open circles indicate the censored observations

Figure 5.3. Observed versus model predicted values for *C. parvum* oocysts inactivation by chlorine dioxide with ozone pretreatment at pH 8.0 and 22°C

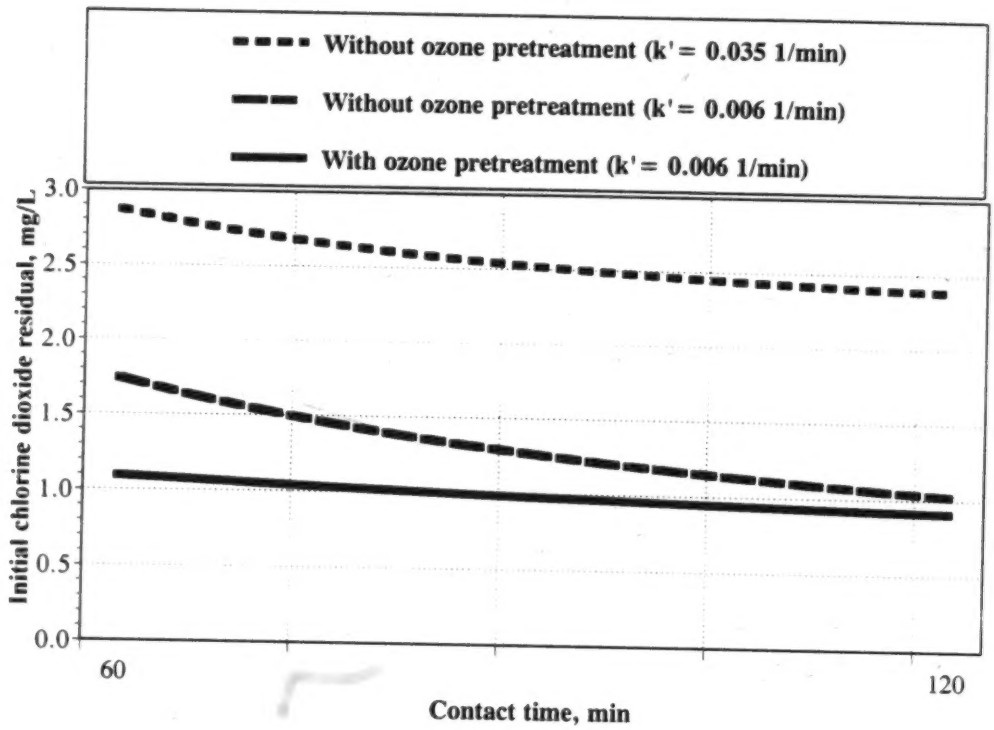


Figure 5.4. Comparison of process design requirements for 3 log-unit(99.9%) inactivation of *C. parvum* oocysts by chlorine dioxide with and without ozone pretreatment (0.7 to 0.8 mg/L ozone for contact time of 4.0 to 5.4 min) at pH 8.0 and temperature 22°C.

Chapter 6

6. General summary and conclusions

6.1 GENERAL OVERVIEW

This study was initiated as a result of the new challenges faced by the drinking water disinfection technology. The findings that, chlorination of drinking water results in harmful disinfection by-products (Bellar et al. 1974; Rook 1974), and the ineffectiveness of traditional disinfection technologies to deal with emerging resistant pathogens such as *C. parvum* (Campbell et al. 1982; Korich et al. 1990b; Lorenzo-Lorenzo et al. 1993; Ransome et al. 1993; Smith et al. 1988; Sundermann et al. 1987), necessiate to re-evaluate the disinfection practices.

Drinking water research community responded to this challenges by looking into new chemical disinfectants which are potentially effective against resistant pathogens at the same time minimizing harmful disinfection by-products. Another parallel approach was to re-evaluate the traditional conservative disinfection design approach (Bellamy et al. 1997; Trussell and Chao 1977) and to look for more rational methods of disinfection design. Integrated rational disinfection design was paid more attention, thereby hoping to minimize the application of quantity of chemical disinfectant while achieving the desired level of disinfection.

Preliminary studies found that ozone or chlorine dioxide have the potential of inactivating *C. parvum* (Finch et al. 1993; Korich et al. 1990b; Peeters et al. 1989), while potentially minimizing some of the undesirable disinfection by-products (Miller et al. 1978; Rav-Acha et al. 1985). Compared to the ozone, minimum data exists for

inactivation of *C. parvum* by chlorine dioxide. Therefore, there is an urgent need for understanding the inactivation of *C. parvum* by chlorine dioxide with regards to the effect of pH, temperature and water quality. Also, there is an urgent need for obtaining kinetic models for inactivation of *C. parvum* by chlorine dioxide which can be integrated with water quality and hydraulics of the contactors to rationally design the disinfection systems. One of the most interesting recent findings indicates that, a greater inactivation can be expected when a combination of disinfectants is used (Finch et al. 1995). Therefore, it is of interest to study the effect of chlorine dioxide in combination with other disinfectants in controlling *C. parvum* in water.

As a result of the above mentioned observations, goals of this study was set as: to determine the efficacy of chlorine dioxide in inactivating oocysts under drinking water disinfection conditions; to determine the effect of pH and temperature on the inactivation of *C. parvum* oocysts by chlorine dioxide; to evaluate the combination of chlorine dioxide and other disinfectants in inactivating *C. parvum* oocysts; and to obtain design graphs for inactivation of *C. parvum* oocysts by chlorine dioxide which will aid engineers in designing chlorine dioxide contactors rationally.

Free chlorine, chlorite, and chlorate may be present as unwanted by-products in water disinfected with chlorine dioxide (Aieta and Berg 1986). These compounds are also oxidants and the effect of these oxidants on *C. parvum* oocysts is of interest. At higher pH, chlorine dioxide dissociates into chlorite and chlorate faster. Therefore, findings that chlorine dioxide is more effective at higher pH in inactivating some bacteria (Junli et al. 1997) and protozoans (Leahy 1985), raised the important question of which oxy-chlorine species are responsible for inactivation. Early studies have shown

that, chlorite and chlorate had no effect on virus (Noss and Olivieri 1985). However, no studies were found which investigate the effect of chlorine dioxide by-products on *C. parvum* oocysts. The results of the Chapter 2, indicate that, under general water treatment disinfection conditions, chlorine dioxide is the only active agent responsible for inactivating *C. parvum* oocysts. These results suggested that, the increased effectiveness of chlorine dioxide at alkaline pH may be a result of structural alterations or some other effect of alkaline pH on oocyst.

As noted in Chapter 1, compared to the literature for inactivation of *C. parvum* oocysts by ozone, there are only few studies which investigate the inactivation of *C. parvum* oocysts by chlorine dioxide (Korich et al. 1990b; Peeters et al. 1989; Ransome et al. 1993). Even though these preliminary findings indicated the potential of chlorine dioxide in inactivating *C. parvum* oocysts, no information was available as to the effect of pH or temperature on inactivation of *C. parvum* oocysts by chlorine dioxide.

Results of this study showed that, chlorine dioxide is more effective at pH 11 than at pH 6 in inactivating *C. parvum* oocysts at 22°C. As noted earlier, it was postulated that, the observed higher inactivation of *C. parvum* oocysts at higher pH results from an effect of pH on the properties of the oocysts but not due to an effect of pH on chlorine dioxide dissociation. Two sets of model parameter were developed for Incomplete Gamma Hom model, which can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide at pH 6 and 11 and at 22°C. Figure 3.4 was presented which can aid engineers in determining chlorine dioxide requirements for inactivation of *C. parvum* oocysts at pH 6 and 11 and at 22°C. Ozone and chlorine

species are less efficient at higher pH. And therefore chlorine dioxide may be an alternative disinfectant for alkaline natural waters or under lime softening conditions.

As expected, it was found in the present study, that chlorine dioxide is significantly more effective at 22°C compared to that at 5°C as described in Chapter 3. Arrhenius activation energy for *C. parvum* inactivation by chlorine dioxide was calculated to be 4.7 kcal/mole. It was also found that, for a 10°C increase of temperature, reaction rate constant of Incomplete Gamma Hom model increases 1.3 fold. Interestingly, another recent study (Gyürék et al. 1997), reported Arrhenius activation energy for *C. parvum* oocysts by ozone to be 3.8 kcal/mole. Indicating that, under otherwise same conditions, ozone is more effective in inactivating *C. parvum* oocysts. More interestingly, same authors reported that, for a 10°C increase of temperature, reaction rate constant of Incomplete Gamma Hom model increases 1.3 fold for ozone inactivation of *C. parvum* oocysts. Therefore, suggesting that, changes in temperature affect ozone and chlorine dioxide in same proportion in inactivating of *C. parvum* oocysts. An experiment conducted to cross validate Arrhenius correction, suggested that Arrhenius correction is applicable to Incomplete Gamma Hom model. Additional set of model parameters were developed for Incomplete Gamma Hom model, which can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide at 5°C and pH 6. A design graph was presented which can aid engineers in determining chlorine dioxide requirements for inactivation of *C. parvum* oocysts at 5°C and at pH 6.

Number of early studies have demonstrated that, simultaneous or sequential application of disinfectants demonstrates synergism between disinfectants resulting higher level of inactivation of micro-organisms (Abad et al. 1994; Bayliss and Waites

1979; Coates and Death 1978; Farkas-Himsley 1964; Gorman et al. 1983; Hall and Sobsey 1992; Katz et al. 1994; Kouame and Haas 1991; Shuval et al. 1995; Straub et al. 1995; Yahya et al. 1992). Recent findings suggest that a similar synergistic effect can be observed with different disinfectants in inactivating *C. parvum* oocysts as well (Finch et al. 1995; Parker and Smith 1993).

Results of the present study, further support that, under some conditions, synergism may exists between chlorine dioxide and chlorine species in inactivating *C. parvum* oocysts. In Chapter 4, the sequential application of chlorine dioxide and chlorine species (namely, free chlorine and monochloramine) was evaluated with respect to their effect in inactivating *C. parvum* oocysts. Two levels of preconditioning with chlorine dioxide were evaluated with each combination. Preliminary experiments were conducted to investigate the effect of pH and temperature on the sequential application of chlorine dioxide and chlorine species. The effect of preconditioning level of chlorine dioxide, and the effect of the level of the secondary disinfectant was evaluated for both combinations. The effect of temperature and pH on the synergistic effect was also presented. Findings of this chapter contribute important information in understanding the synergism between chlorine dioxide and chlorine species in inactivating *C. parvum* oocysts.

Specific findings were that, both chlorine dioxide followed by free chlorine and monochloramine demonstrate synergism between these oxidants in the pH range of 6 to 8 at 22°C. The results further suggested that there may be an optimum level of chlorine dioxide preconditioning above which there is no added advantage due to synergism. Preliminary data further indicated that, synergism is less pronounced at pH 11 at 22°C

compared to that at pH 6 to 8. Preliminary experiments did not show any synergism at 5°C between pH 6 to 8 with both combinations.

Sequential application of ozone and chlorine dioxide was also evaluated at pH 8 and 22°C. It was found that, significantly more inactivation of *C. parvum* oocysts can be achieved due to the synergism between ozone and chlorine dioxide. Two sets of model parameter were developed for integral Hom model, which can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide with and without ozone preconditioning at pH 8 and 22°C.

In summary, the results of the present study indicate chlorine dioxide was found to be a potent disinfectant in controlling *C. parvum* oocysts in water. It was also found that, chlorine dioxide is more effective in inactivating *C. parvum* oocysts at alkaline pH. Temperature effect was found to be similar in magnitude to that of *C. parvum* oocysts inactivation by ozone. Chlorine dioxide followed by chlorine species and ozone followed by chlorine dioxide may have the potential of being a strong barrier in controlling *C. parvum* in water at warm temperatures.

6.2 SPECIFIC CONCLUSIONS OF THIS STUDY

1. chlorine dioxide was an effective disinfectant in controlling *C. parvum* oocysts in drinking water.
2. aqueous chlorine, sodium thiosulfate, chlorite, chlorate and the reaction by-products of chlorite or chlorate when reduced with sodium thiosulfate had no detectable effect on *C. parvum* oocysts. These results suggest that chlorine dioxide is the active agent

responsible for inactivation of *C. parvum* oocysts under typical conditions of chlorine dioxide application for drinking water disinfection.

3. chlorine dioxide was found to be significantly more effective at pH 11 compared to pH 6 in inactivating *C. parvum* oocysts at 22°C. Since it was observed that, chlorine dioxide dissociation by-products have no cysticidal effect, the effect of pH is more likely due to changes in *C. parvum* oocysts which make it more susceptible to chlorine dioxide at higher pH.
4. Data reported by Korich et al, (1990a; 1990b)and Peeters et al, (1989) on inactivation of *C. parvum* oocysts apparently overestimates the effect of chlorine dioxide compared to the results of the present study.
5. Incomplete Gamma-Hom model, which accounts for the disinfectant decay, can adequately describe the inactivation of *C. parvum* oocysts by chlorine dioxide under different pH and temperature conditions.
6. inactivation of *C. parvum* oocysts by chlorine dioxide is non-linear with respect to concentration and contact time. Therefore, the use of CT criterion in evaluating disinfection credits for *C. parvum* oocysts inactivation by chlorine dioxide is not recommended.
7. chlorine dioxide was found to be significantly more effective at 22°C compared to 5°C in inactivating *C. parvum* oocysts at pH 6. Arrhenius Activation energy for inactivation of *C. parvum* oocysts by chlorine dioxide was found to be 4.7 kcal/mole. It was also found that, for every 10°C increase of temperature, reaction rate constant of Incomplete Gamma Hom model increases 1.3 fold.

8. sequential disinfection with chlorine dioxide followed by free chlorine or monochloramine showed a significantly more inactivation of *C. parvum* oocysts due to the synergistic effect between chlorine dioxide and free chlorine or monochloramine at pH 6.0 and 8.0 at 22 °C.
9. there may exist an optimum level of preconditioning with chlorine dioxide above which there is no increase in synergism, even though the total inactivation is increased.
10. level of synergism is increased with the CT level of secondary disinfectant in case of free chlorine. Limited data showed that apparently synergism is not increased with respect to monochloramine CT level.
11. lower level of synergism was observed between chlorine dioxide and chlorine species at pH 11, compared to pH 6 and pH 8.
12. preliminary results show that, there is no synergism, at 5°C at pH 6 and 8, between chlorine dioxide and chlorine species investigated.
13. synergism between chlorine dioxide and chlorine species is a complex process and more studies are needed to understand the process more clearly.
14. the sequential treatment of *C. parvum* oocysts by ozone followed by chlorine dioxide resulted in additional inactivation due to the synergism of the two disinfectants.
15. with ozone pretreatment, chlorine dioxide may effectively be used to achieve inactivation of *C. parvum* oocysts up to 3 log-units while meeting current U.S. Environmental Protection Agency regulatory limits for chlorine dioxide application.
16. combination of ozone and chlorine dioxide may have potential in controlling waterborne parasites and hence is a candidate for further investigation.

6.3 LIMITATIONS OF THE STUDY AND RECCOMMENDATIONS

All experiments were conducted in high quality laboratory water, and therefore the effect of different qualities of natural water was not investigated. However, as explained in Chapters 3, water quality may be represented by the chlorine dioxide decay and can be incorporated into the kinetic models reported in this study. It will be of interest to test these models with various natural water qualities to check the validity of the proposed kinetic models.

Because of the high cost and labor involved with the animal infection procedure, the kinetic models were developed with limited number of data. Therefore, it is highly recommended that, cross-validation trials be conducted to validate the proposed kinetic models.

Effect of very low temperatures such as (1°C) was not evaluated in this study. As some of the water treatment plants operates at these temperatures, the inactivation of *C. parvum* oocysts at these temperatures is of interest. At near freezing temperatures, the matrix of water molecules starts to change and the structure of oocysts wall also may be changed. As a result, application of kinetic models may also be affected. Therefore, development of kinetic models and specially a feasibility study of application of Arrhenius temperature correction at these near freezing temperatures are of great interest.

Only preliminary experiments were conduct to evaluate the effect of low temperatures on the synergism between chlorine dioxide and chlorine species in the present study, and therefore a definitive study to investigate the low temperatures on inactivating *C. parvum* oocysts by sequential application of chlorine dioxide followed by

chlorine species is highly recommended. Due to similar reasons, a definitive study to evaluate the effect of pH on the synergism between chlorine dioxide and chlorine species in inactivating *C. parvum* oocysts is also highly recommended.

When evaluating ozone and chlorine dioxide combinations, only one ozone preconditioning level was investigated in the present study. Therefore, development of kinetic models for different level of ozone preconditioning levels will help understand and to optimize this very promising combination. Also, ozone and chlorine dioxide were not investigated at low temperatures.

All the disinfectant studies with combinations of disinfectants, stronger oxidant was applied first followed by weaker oxidant. It is of interest to study the effect of simultaneous application of oxidants under identical conditions to help evaluate the hypothesis of sequential disinfection.

All chlorine dioxide followed by free chlorine studies were done at one pH for both oxidants. As chlorine dioxide is more effective at higher pH and free chlorine is more effective at low pH, it would be of interest to study the sequential application of chlorine dioxide at alkaline pH (for example pH 11) followed by application of free chlorine at acidic pH (for example pH 6). A similar experiments can be conducted with ozone followed by chlorine dioxide with ozone at lower pH followed by chlorine dioxide at higher pH.

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Appendix A

Disinfection methods

Laboratory water

Due to the nature of the experiments, it was desirable tc minimize as many sources of experimental error as possible. Since the data from this research will be used widely, it was desirable to have a uniform, reproducible water quality that can be reproduced on demand. For this reason, we used deionized water that was obtained from a purification system Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm).

Procedure for making glassware ozone demand free:

Any glassware that will come in contact with disinfectant or reagents were made ozone demand free. This includes bottles for phosphate buffer, trial reaction vessels, Eppendorf® and Oxford® pipettes of all sizes, stir bars, and miscellaneous containers.

Ozone was generated using procedures described in Chapter 5. Briefly, a container was filled with Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm). Then stir bar was added to the container. The container was then placed on a stir box in the fume hood. The stir box was turned on so that the ozone will have good contact with the water. Using the sparger, ozone was bubbled into the solution for 10 minutes per each liter of water in the container to saturate the water with ozone. After that any container to be made ozone demand free was filled with this ozonated Elga® (Fisher Scientific, Pittsburgh, Pennsylvania) deionized water (resistivity at least 18 MΩ/cm) water. Then a lid was placed on each container (a piece of aluminum foil), and the container was soaked for at least thirty

minutes. After the ozone contact time, water was removed and the lid was replaced and put into a drying oven that is greater than 65°C for a sufficiently long time(generally overnight).

Preparation of ozone demand free buffers

The standard 0.05 M phosphate buffer was prepared using potassium dihydrogen orthophosphate and disodium hydrogen phosphate (BDH Inc. AnalaR grade). The resulting solution was made oxidant demand-free by bubbling ozone through the solution for at least 30 minutes, followed by boiling for at least 10 minutes and then was cooled in a clean air hood. The resulting solution was found to be ozone, chlorine dioxide and chlorine demand-free.

Preperation of pre formed monochloramine solutions

Monochloramine solution was made by diluting a 1000 mg/L free chlorine stock solution down so that it is 300 mg/L as chlorine (in a ozone demand free 100 mL volumetric flask with ozone demand free water). Then a 10 mL of ammonium chloride stock solution containing 3.8187g/L of ammonium chloride (which cannot be made ozone demand free) was diluted to 100 mL. This diluted stock gives a nitrogen content of 100 mg/L. In a ozone demand free 250 mL bottle, the 300 mg/L chlorine stock was added to the diluted ammonium chloride stock. The order that the reagents are added is important. Always chlorine was added to the ammonium chloride. Following 30 minutes of stirring, the free chlorine was found to be converted to monochloramine, and the concentration of the solution was around 150 mg/L.

General disinfection procedure.

A defined disinfection protocol was used in all trials (Haas et al. 1993). Each concentration and time combination was conducted as an individual trial and the trials were randomized using the procedures of Box et al. (1978) and Draper and Smith (1981). The concentration of the stock disinfectant solution was measured immediately before the experimental trial was performed. A calculated volume of stock solution for the required initial disinfectant dose was then added to the reactor containing the oocysts using a mass-calibrated pipette. In the case of ozone and chlorine dioxide, the residual concentration was monitored continuously by UV absorbance at 260 and 360 nm, respectively.

At the end of the contact time remaining oxidants were neutralized using reagent grade chemicals. Ozone was neutralized using 1M sodium formate, chlorine dioxide was neutralized using 0.1 N sodium thiosulfate and chlorine species neutralized with 1M sodium sulfite. Excess levels of these reducing agents have been demonstrated neither reduce nor to enhance oocyst infectivity. An additional reactor served as a negative control in all experiments receiving the same dose of reducing agent. The contents of the entire reactor vessel were then concentrated by centrifugation for subsequent viability analysis.

Ozone disinfection.

The 0.05 M phosphate buffer was prepared using potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate mixed to provide the required pH (BDH Inc. 'AnalaR' grade). The solution was made ozone demand-free following procedures described earlier, and the ozone generated using the procedures of described in Chapter 5. The parasites from the ozonated vessel and control vessel were

concentrated by centrifugation at 600 x *g* in a swinging-bucket centrifuge (GR4.11; Jouan) at 4°C for 10 min. Supernatant was aspirated from each of the 250 mL vessels leaving approximately 1 mL of oocyst suspension. The parasite concentration in both reactors was determined by counting with a hemocytometer and the percent recoveries were calculated for ozone treated and control samples.

Chlorine disinfection.

Free chlorine experimental protocols followed those recommended by Haas *et al.* (1993). Free chlorine stock solutions were prepared prior to each experiment using sodium hypochlorite. Free chlorine residuals were measured using the DPD colorimetric procedure (Greenberg, Clesceri & Eaton, 1992). At the end of the contact time, 50 mL of 1 M sodium sulfite (Na_2SO_3) was used to neutralize residual chlorine. Sodium sulfite was similarly applied to an additional ozone demand-free vessel containing 10 X 10^6 parasites to serve as a control.

Chlorine dioxide disinfection.

Chlorine dioxide stock solution was generated by a patented chlorine dioxide generator (CDG Technology, Inc.; New York, NY), where dilute chlorine gas in a nitrogen carrier is passed through a packed bed of sodium chlorite. The gas flow rate was adjusted so that all of the chlorine gas was consumed in the reaction column. The process gas was captured in a 500 mL gas absorption flask containing deionized water. The concentration of chlorine dioxide in the absorption flask was in excess of 9,000 mg/L. No chlorine residual has been detected to date and the solution was not used if chlorine was present. The concentration of free chlorine, chlorate and chlorite was determined using amperometric titration (Greenberg et al. 1992). Since temperature and

ultraviolet radiation affect solution stability, chlorine dioxide losses were minimized by storing the stock solution in a dark refrigerator. The stock solution was checked periodically and was discarded when the concentration dropped by 5 per cent of the initial residual.

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Appendix B

Parasite methods

Parasite.

The strain of *C. parvum* oocysts used in this study comes from Dr. Frank W. Schaefer III (EMSL, U.S. Environmental Protection Agency, Cincinnati, Ohio). Dr. Schaefer obtained his strain from Dr. Charles R. Sterling (Department of Veterinary Science, University of Arizona, Tucson, Arizona) who originally obtained this isolate from Dr. Harley Moon (National Animal Disease Center, Ames, Iowa).

Production and purification of oocysts.

The oocysts used in this study were produced in male neonatal Holstein calves (*Bos tauris*) using a modification of the methods described elsewhere (Musial et al. 1987). Calves obtained at birth, were given up to 2 L of colostrum from a bottle. Within 12 h the animals were dosed from a bottle containing 2×10^8 *C. parvum* oocysts suspended in 2 L of milk replacer. The calves were maintained on a diet of 1 part milk replacer and 1 part electrolyte solution during fecal collection.

At the onset of scouring, the feces were collected in tap water and sequentially passed through 10, 20, 60, 100, 200 and 400 mesh sieves (ATM Test Sieves, Inc., Milwaukee, WI) by agitating and washing the sieves with 0.01% (v/v) aqueous Tween 20. Concentration of the particulate from the sieved feces was done by centrifugation at 1,100 x *g* for 10 min.

The oocysts were purified by concentrating the parasites using sucrose flotation and cesium chloride gradient ultracentrifugation. A fifty mL conical centrifuge tube was filled with 30 mL of 2 M sucrose solution onto which 5 to 9 mL of emulsified feces were

layered. The tubes were mixed by inverting 10 times overlaid with water and centrifuged at 800 x *g* for 10 min. at 4 C. The oocysts found at the water-sucrose interface were removed using a pipette and diluted 5 times using deionized (Milli-Q®) water containing 0.01% Tween-20. The oocysts were then washed 3x in deionized water containing 0.01% Tween-20 at 2,800 x *g* for 20 min. at 4 C. After the final wash, the supernatant was removed and the oocyst pellet stored at 4 C prior to the final purification of the parasites using Cesium chloride gradient centrifugation. The Cesium chloride gradient was prepared in a 40 mL Beckman polyallomer ultracentrifuge tube. The gradient consisted of a bottom layer (9 mL of 1.4 g/mL Cesium chloride), middle layer (9 mL of 1.1 g/mL Cesium chloride) and a top layer (9 mL of 1.05 g/mL Cesium chloride). Approximately 3 mL of the parasite material was gently layered on top of the Cesium chloride gradient and centrifuged at 16,000 x *g* for 60 min. using a swinging-bucket rotor (SW-28) at the slow brake setting (Beckman L7-55 ultracentrifuge). After centrifugation, the top dense band (out of three formed following ultracentrifugation) contained the purified oocysts which were removed using a pipette and placed in 15 mL polypropylene snap cap tubes. The tubes were filled with deionized water containing Tween-20 and washed 2x by centrifugation at 14,500 x *g* for 10 min. using a fixed-angle SS-34 rotor of a high speed centrifuge (Sorval, RC5-B centrifuge). After the final washing step, the oocysts were suspended in 0.01% Tween-20 containing 100 units penicillin per mL, 100 µg streptomycin per mL, and 100 µg gentamycin per mL and were stored at 4°C. The oocyst concentration of the suspension was determined by counting the parasites using a hemocytometer. The typical concentration of oocysts in the stock suspension was 1.5 to 2. 5 x 10^8 per mL. In our studies, *C. parvum* oocysts

were never exposed to 2.5 per cent potassium dichromate or sodium hypochlorite as is commonly done. The reason for this is that under natural conditions, oocysts found in water samples are not exposed to these chemicals. Consequently, oocysts preserved in potassium dichromate or sodium hypochlorite may not be representative of the conditions that occur in nature.

Animal Infectivity.

A neonatal mouse model was used to evaluate infectivity of *C. parvum* (Ernest et al. 1986; Finch et al. 1993) . Breeding pairs of outbred CD-1 mice were obtained from the Charles River Breeding Laboratories (St. Constant, Quebec, Canada). The animals were given food and water *ad libitum* and were housed in cages with covers fitted with a 0.22 µm filter in a specific pathogen-free (P-2 level) animal facility.

Oocyst doses were prepared from the stock suspension by serial dilution to obtain the required dose. The actual dose given to the mice was determined from a hemocytometer count of the stock suspension and the dilution factor. The mice were orally inoculated 4 d after birth using a micropipetter with a known numbers of oocysts suspended in 5 to 10 µL of deionized water.

The infectivity of the oocysts was determined 7 d after infection. The mice were killed by cervical dislocation and the lower half of the small intestine, the cesium and the colon removed and placed in 10 mL of deionized water. The intestines were homogenized for 10 s in a Sorvall® Omni-Mixer and washed three times in deionized water containing 0.01% Tween-20 at 2,000 x *g* for 15 min. After centrifugation, the supernatant was discarded and 10 mL of Sheather's sugar solution was added to the pellet and centrifuged at 1,000 x *g* for 10 min. A few drops from the surface of the

suspension were removed and examined with differential interference contrast (DIC) microscopy at 400x. Mice were scored either positive or negative for oocysts after examination of the slide. The total oocysts loads were not estimated in the exposed mice.

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Appendix C

Calculation of chlorine dioxide decay rate constant.

A measured volume of chlorine dioxide from a concentrated stock solution was added to the reactor, and the reactor content was agitated using a Teflon®-coated magnetic stir bar. The reactor (containing oocysts) was continuously sampled in a closed loop at a flow rate of 8 mL/min. The sample was carried through a short piece of small-diameter Teflon® tubing to a 35 µl flow cell with a light path of 1 cm, and the concentration of chlorine dioxide in aqueous solution was continuously monitored using ultraviolet spectrophotometry at 360 nm using a molar absorptivity of 1,250 $M^{-1} \cdot cm^{-1}$ (Gordon et al. 1992). At the end of the contact time, residual chlorine dioxide was neutralized with sodium thiosulfate.

The difference between the initial baseline absorbance and measured absorbances over the time was multiplied by the factor of 53.98 to obtain the observed residual chlorine dioxide concentration. Predicted concentration (C) at any time is given by the Equation C.1.

$$C = C_0 Exp(-k'*t) \qquad (C.1)$$

where C_o is the initial observed concentration, k' first order chlorine dioxide decay rate constant, and t is the time elapsed.

The absorbance profile of Trial 760 for *C. parvum* inactivation by chlorine dioxide at pH 6 and 22°C is given in Table C.1. The same format in Table C.1 was used to estimate the first order chlorine dioxide decay rate constant (k') using solver routine in Microsoft® Excel (Microsoft Canada, Mississauga, Ontario).

Table C.1. The time absorbance profile for Trial # 760 and estimation of first order chlorine dioxide decay rate constant.

Time, s	Absorbance	Time, min.	Conc.(Obs), mg/L	Conc.(pred), mg/L	k' (1/min.)	SS*
160	0.05162	0.00	2.10	2.10	0.0037	0.0015
340	0.05104	3.00	2.06	2.07		
708	0.05017	9.13	2.02	2.03		
1364	0.04871	20.07	1.94	1.95		
2284	0.04628	35.40	1.81	1.84		
3220	0.04468	51.00	1.72	1.73		
4704	0.04225	75.73	1.59	1.58		
5968	0.04007	96.80	1.47	1.46		
7152	0.03819	116.53	1.37	1.36		
Baseline	0.0128					

*SS- Residual sum of squares

The observed and first order model (Equation C.1) predicted residual concentration profile is graphically shown in Figure C.1.

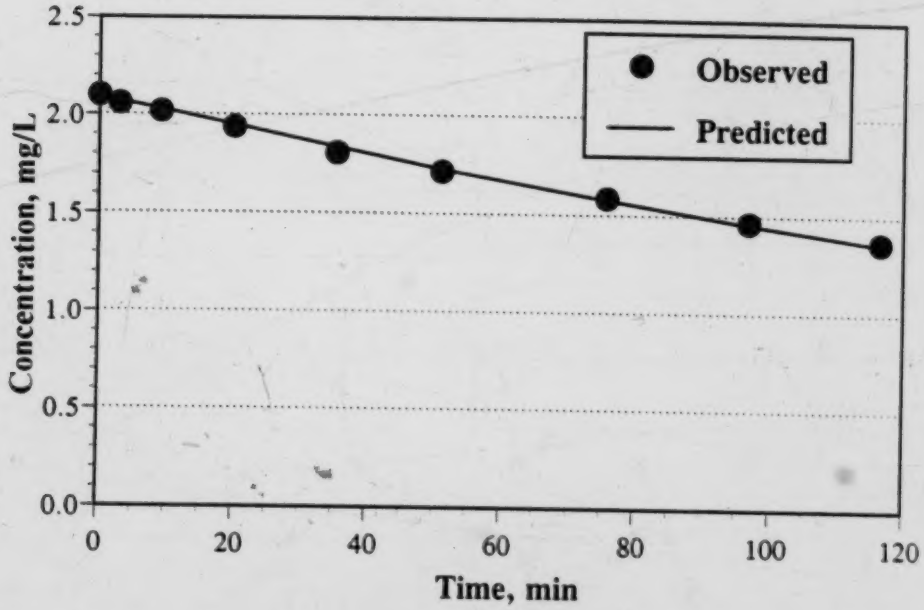

Figure C.1. Observed and first order model predicted chlorine dioxide residual profile for trial 760 (*C. parvum* inactivation at pH 6 and 22°C)

Appendix D

Mice infectivity results

Table D.1. Summary of data used to estimate the parameters for *C. parvum*/CD-1 mice dose response models for Batch 17, 18 and 19

Oocysts batch number	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Observed proportion infected	logit model predicted proportion infected
Batch 17	25	20	7	0.35	0.40
	50	15	11	0.73	0.60
	100	25	18	0.72	0.80
	200	23	22	0.96	0.90
Batch 18	35	17	6	0.35	0.30
	70	20	10	0.50	0.60
	140	19	15	0.80	0.80
Batch 19	35	19	5	0.30	0.21
	70	19	9	0.47	0.55
	140	20	17	0.85	0.84
	280	10	10	1.00	0.96

Table D.2. Summary of data used to estimate the parameters for *C. parvum*/CD-1 mice dose response models for Batch 20 and 21

Oocysts batch number	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Observed proportion infected	logit model predicted proportion infected
Batch 20	25	10	3	0.30	0.27
	70	10	7	0.70	0.70
	100	10	7	0.70	0.82
	140	10	10	1.00	0.89
	200	8	7	0.88	0.94
	280	10	10	1.00	0.97
Batch 21	25	10	2	0.20	0.20
	50	9	4	0.44	0.45
	100	10	6	0.60	0.73
	140	10	10	1.00	0.83
	200	10	10	1.00	0.90
	280	10	8	0.80	0.94

Table D.3. Summary of data used to estimate the parameters for *C. parvum*/CD-1 mice dose response models for Batch 22 and 23

Oocysts batch number	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Observed proportion infected	logit model predicted proportion infected
Batch 22	70	10	3	0.30	0.30
	140	10	6	0.60	0.72
	280	9	8	0.89	0.94
	70	9	3	0.33	0.30
	140	10	8	0.80	0.72
	280	10	10	1.00	0.94
	560	7	7	1.00	0.99
Batch 23	35	9	2	0.22	0.31
	70	8	5	0.63	0.49
	140	7	6	0.86	0.66
	35	9	4	0.44	0.31
	70	8	2	0.25	0.49
	140	9	5	0.56	0.66
	280	10	7	0.70	0.80

Table D.4. Summary of mice response data for inactivation of *C. parvum* by chlorine dioxide at pH 6 and 22°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Logit model calculated inactivation, log-units	Observed control inactivation, log-units	Observed net inactivation, log-units
760	1200	10	4	20	2.0	0.2	1.8
	12000	10	5				
768	400	8	3	20	1.7	0.2	1.5
	4000	8	2				
770	1200	8	1	20	2.1	0.2	1.9
	12000	8	6				
772	40	7	3	20	0.4	0.2	0.2
	400	7	5				
774	125000	10	1	20	4.0	0.2	3.8
	1250000	10	9				
776	40	10	6	20	0.0	0.2	0.0
	400	10	10				
863	400000	10	0	21	>5.0	0.2	>4.8
	1460000	10	0				

Table D.5. Summary of mice response data for inactivation of *C. parvum* by chlorine dioxide at pH 11 and 22°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Logit model calculated inactivation, log-units	Observed control inactivation, log-units	Observed net inactivation, log-units
778	400000	9	8	20	3.5	0.1	3.4
	1600000	8	8				
780	400	9	0	20	2.1	0.1	2.0
	4000	8	3				
784	40	10	7	20	0.1	0.2	0.0
	400	10	9				
786	40	10	5	20	0.0	0.2	0.0
	400	10	10				
793	1200	10	0	20	2.7	0.2	2.5
	12000	10	3				
795	400000	10	6	20	3.8	0.2	3.6
	940000	10	9				
797	1400	10	1	20	2.0	0.2	1.8
	14000	10	9				
867	400000	10	2	21	4.8	0.1	4.7
	1460000	10	5				

Table D.6. Summary of mice response data for inactivation of *C. parvum* by chlorine dioxide at pH 6 and 5°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Observed net inactivation, log-units
919	400	8	3	22	0.6
	4000	8	8		
923	40	10	5	22	0.2
	400	8	7		
943	200	7	1	23	2.4
	2000	8	2		
945	600	7	0	23	>3.4
	6000	6	0		
947	40	7	2	20	1.0
	400	9	8		
949	150	7	0	23	2.2
	1500	4	2		
951	40000	10	0	23	>4.2
	400000	9	0		
959	40000	10	0	23	>4.0
	400000	8	0		

Table D.7. Summary of mice response data for inactivation of *C. parvum* by sequential application of chlorine dioxide followed by free chlorine at pH 8 and 22°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Observed net inactivation, log-units
595	7500	10	8	17	2.0
	75000	9	9		
628	400	10	1	17	1.7
	4000	9	8		
756	12500	9	5	20	2.3
	125000	9	9		
758	100000	10	8	20	2.9
	1000000	7	7		
843	30000	10	1	21	3.7
	300000	8	7		
845	10000	10	8	21	2.4
	100000	6	6		
815	100	10	5	21	1.1
	1000	10	8		
817	300	9	5	21	1.6
	3000	10	7		
821	600	5	1	21	2.1
	6000	8	6		
839	600	10	7	21	1.7
	6000	10	10		
841	700	10	0	21	2.7
	7000	10	4		

Table D.8. Summary of mice response data for inactivation of *C. parvum* by sequential application of chlorine dioxide followed by monochloramine at pH 8 and 22°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Observed net inactivation, log-units
724	2000	10	3	19	2.2
	20000	9	7		
728	12000	8	6	19	2.6
	120000	10	9		
732	50000	4	3	19	2.9
	500000	5	5		
738	5000	10	0	19	2.8
	50000	10	5		
742	4000	6	0	19	2.5
	40000	7	5		
744	12000	8	1	19	3.1
	120000	7	1		
825	200	8	1	21	1.8
	2000	9	5		
827	100	10	2	21	1.5
	1000	10	3		
829	100	10	0	21	2.3
	1000	10	1		
833	500	8	2	21	2.0
	5000	10	7		
835	600	10	0	21	2.8
	6000	9	2		

Table D.9. Summary of mice response data for inactivation of *C. parvum* by sequential application of ozone followed by chlorine dioxide at pH 8 and 22°C.

Experimental trial	Inoculum, oocysts	Number of mice inoculated	Number of infected mice	Relevant oocyst batch	Observed net inactivation, log-units
630	3800	7	0	17	2.5
	38000	9	8		
705	18000	10	0	18	3.6
	180000	9	5		
707	10000	10	0	18	3.4
	100000	9	5		
715	1500	7	1	18	2.0
	15000	9	8		
717	150000	5	0	18	>4.8
	1500000	3	0		

Appendix E. 90% joint confidence regions for "m" and "n" (conditional for optimal "k") for I.G.H models

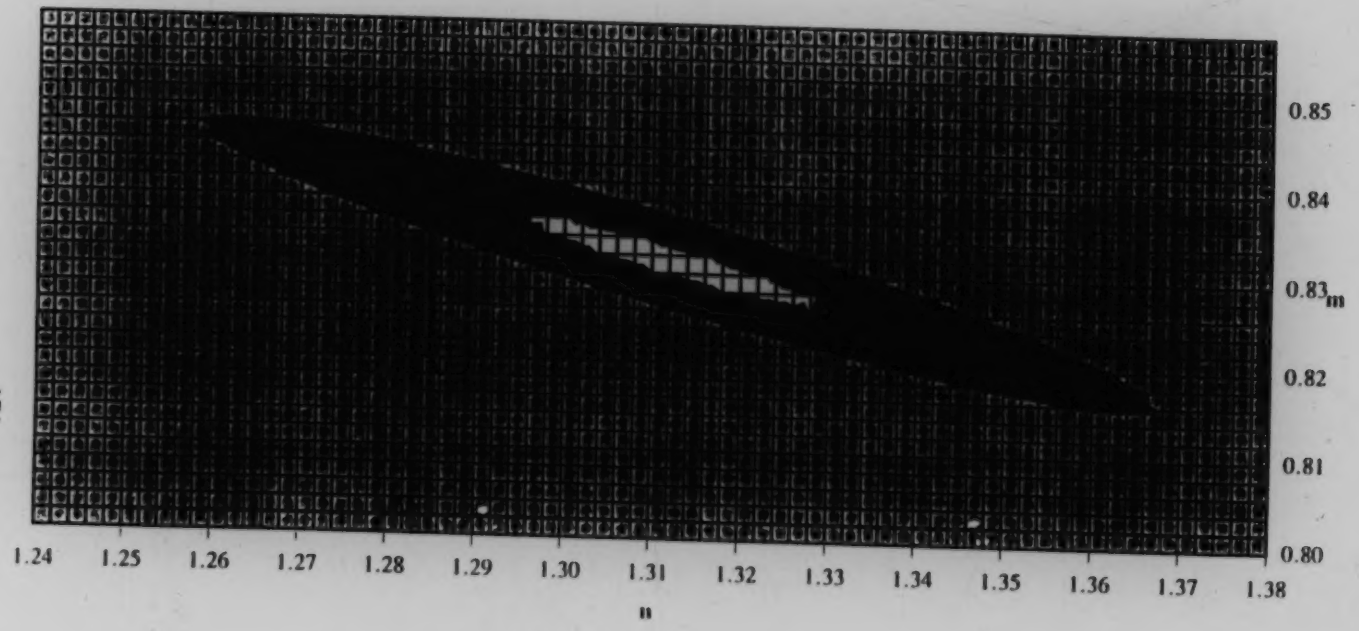

Figure E.1. Approximate 90% joint confidence regions for "m" and "n" (conditional for optimal "k") for I.G.H model for inactivation of *C. parvum* by chlorine dioxide at pH 6 and 22°C.

Figure E.2. Approximate 90% joint confidence regions for "m" and "n" (conditional for optimal "k") for I.G.II model for inactivation of *C. parvum* by chlorine dioxide at pH 6 and 5°C.

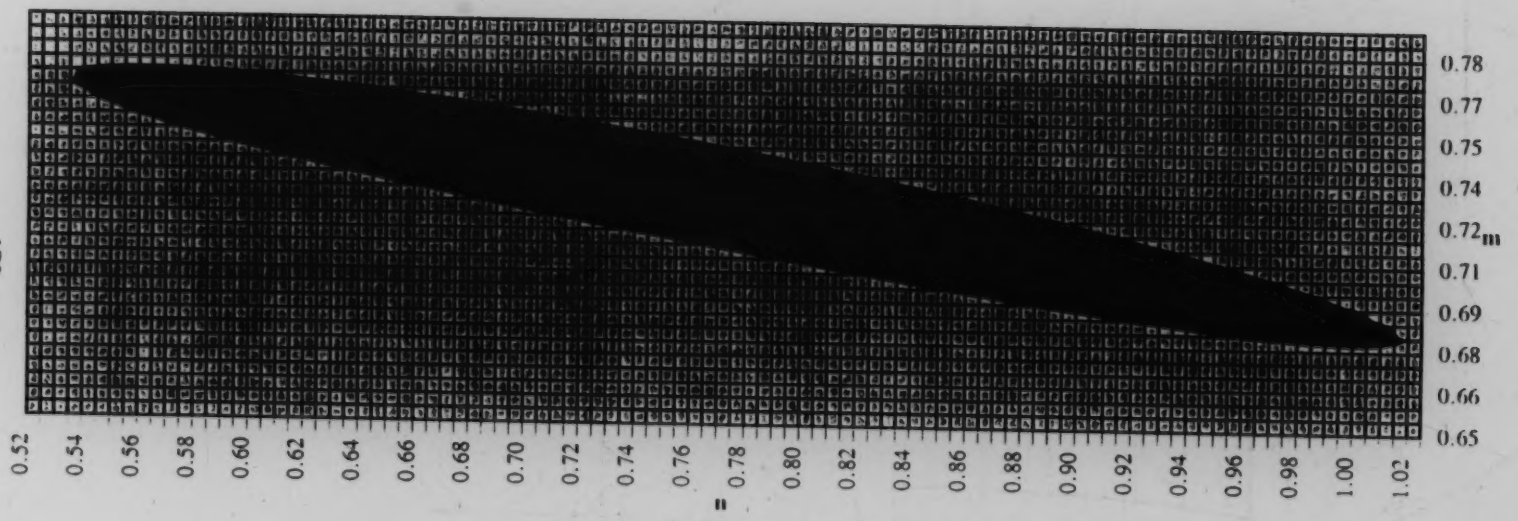

Figure E.3. Approximate 90% joint confidence regions for "m" and "n" (conditional for optimal "k") for I.G.H model for inactivation of *C. parvum* by chlorine dioxide at pH 11 and 22°C.

Appendix F. Residual plots

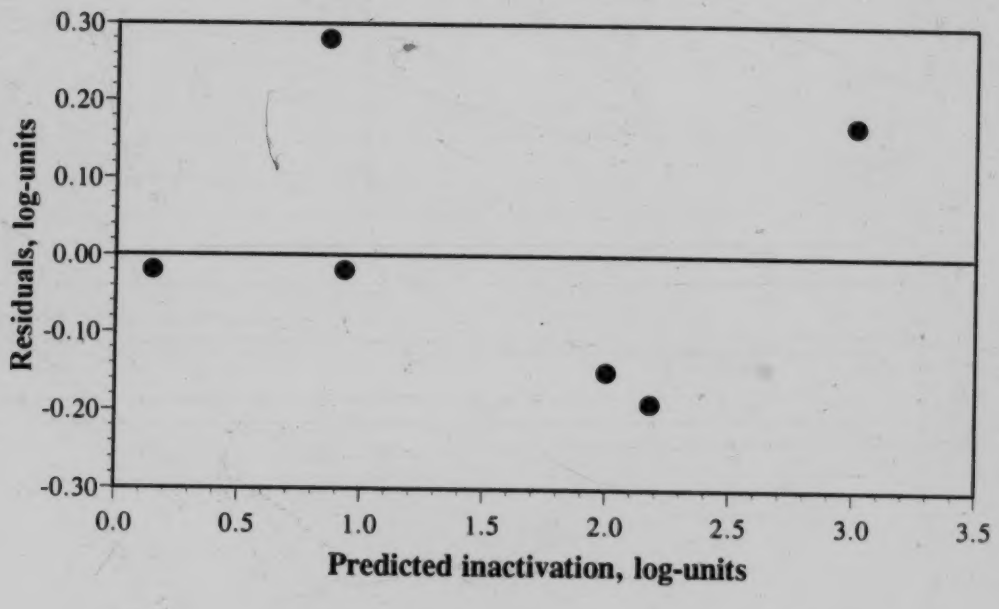

Figure F.1. Residual vs predicted inactivation plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 6 and 5°C.

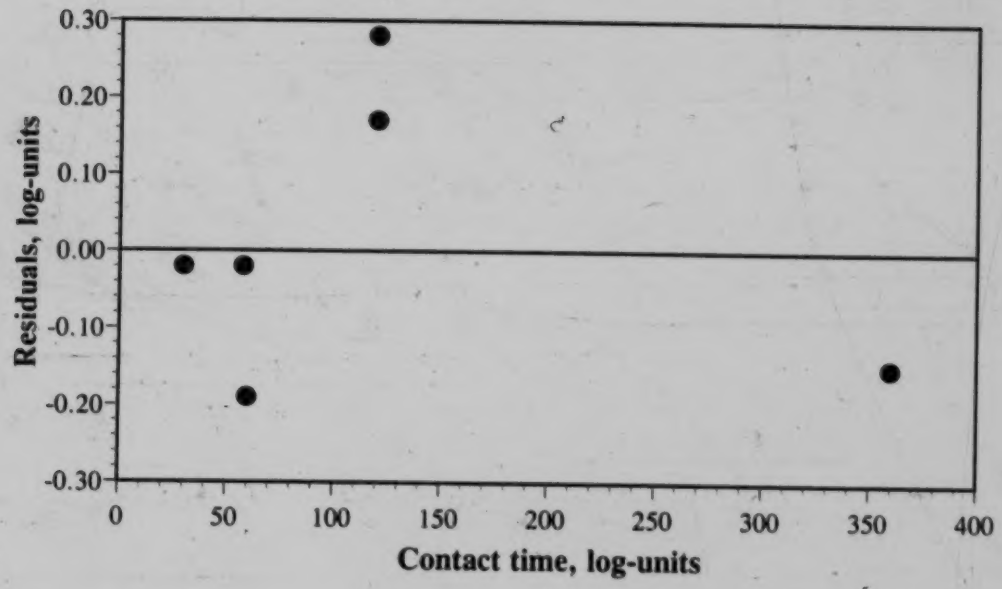

Figure F.2. Residual vs contact time plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 6 and 5°C.

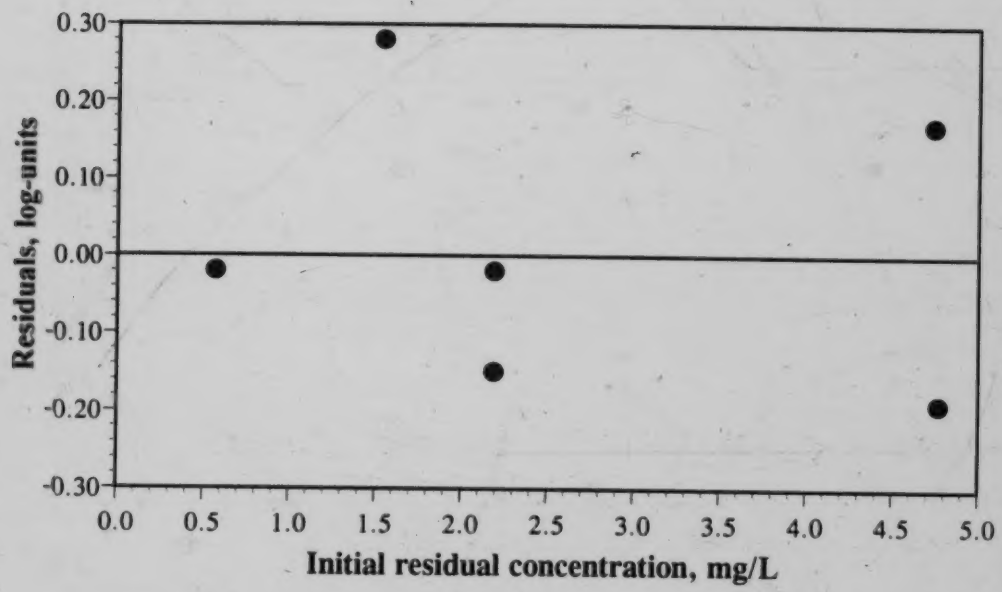

Figure F.3. Residual vs initial residual concentration plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 6 and 5°C.

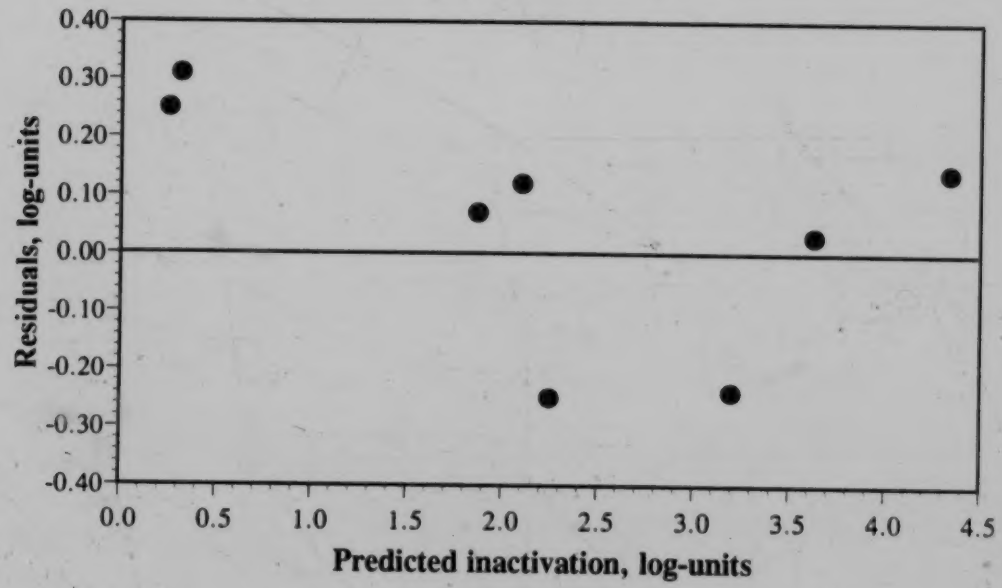

Figure F.4. Residual vs predicted inactivation plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 11 and 22°C.

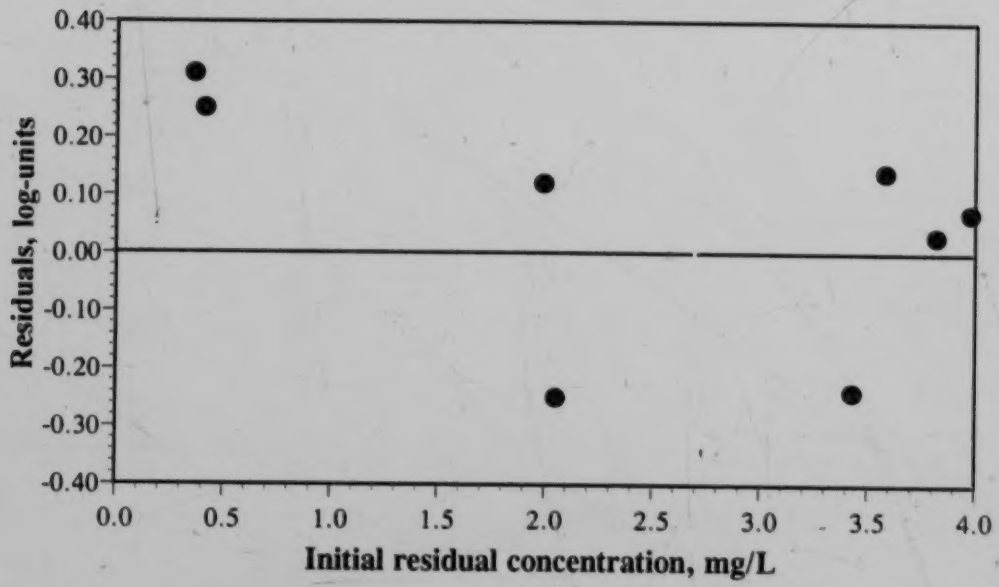

Figure F.5. Residual vs initial residual concentration plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 11 and 22°C.

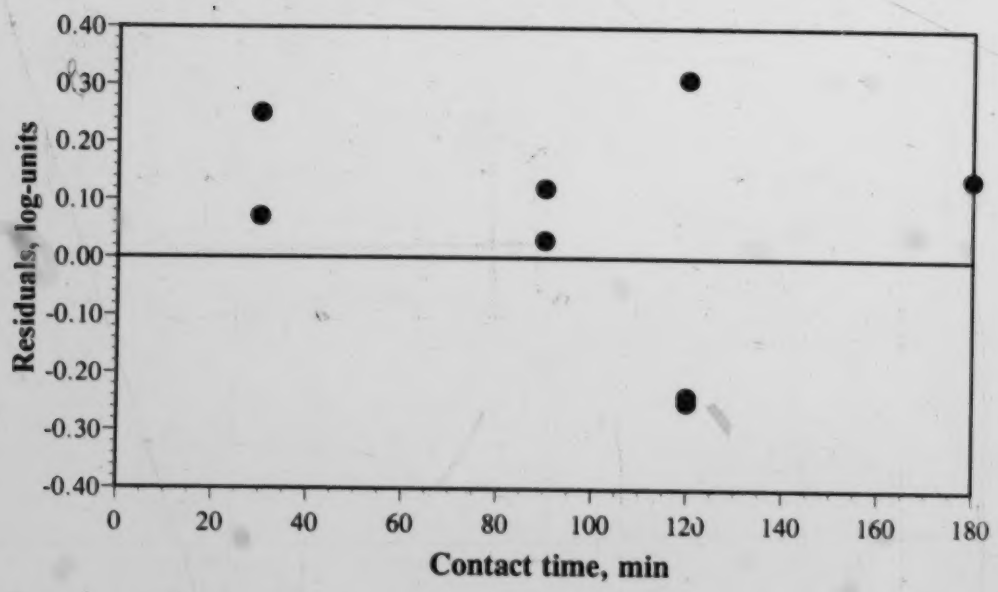

Figure F.6. Residual vs contact time plot of I.G.H. model for *C. parvum* inactivation by chlorine dioxide at pH 11 and 22°C.

Appendix G. Physical and thermodynamic properties of chlorine dioxide

Chlorine dioxide is a greenish yellow gas having a characteristic odor that is distinctive from that of chlorine. Liquid chlorine dioxide has a deep red color and is explosive at temperatures above -40°C. Selected physical and thermodynamic properties are given in Table G.1. Chlorine dioxide exists as a free-radical monomer. The aqueous ultraviolet absorption spectrum of chlorine dioxide has a broad absorption band. The maximum is near 360 nm and the molar extinction coefficient, given as 1150 $M^{-1}.cm^{-1}$, is more accurately valued at 1250 $M^{-1}.cm^{-1}$. The extinction coefficient is independent of temperature from 25 to 50°C, and from acid concentrations ranging from 0.2-4 N, ionic strength, 2-4 M, and chloride ion concentration up to 0.3 M. The chlorine dioxide absorption spectrum in aqueous or organic solutions is the same as the gas phase spectrum. Chlorine dioxide is soluble in water, forming a yellow to yellow-green color solution that is quite stable if kept cool and in the dark.

Table G.1. Physical and thermodynamic properties of chlorine dioxide (Kaczur and Cawlfield 1993)

Property	Value
Molecular weight	67.452 g
Critical temperature	465 K
Critical pressure	8621.6 kPa
Melting point	213.55 K
Triple point temperature	213.55 K
Triple point pressure	1.2544 kPa
Boiling point (at 101.3 kPa)	284.05 K
Liquid molar volume	4.1852×10^{-2} m^3/kmol
Density of liquid (at 0°C)	1.773 g/mL
Ideal gas heat of formation	102.5 kJ/mol
Ideal gas Gibbs energy of formation	120.5 kJ/mol
Ideal gas entropy	0.257 kJ/(mol.K)
Standard net heat of combustion (gas)	-102.5 kJ/mol
Vapor pressure (0°C)	64.419 kPa
Latent heat of vaporization	26.937 kJ/mol
Ideal gas heat capacity	0.0408 kJ/(mol.K)

References

Kaczur, J.J., and D.W. Cawlfield. 1993. Chlorous Acid Chlorites, and Chlorine Dioxide. In *Kirk-Othmer Encyclopedia of Chemical Technology*. Volume 5, pp. 968-997. New York, NY: John Wiley & Sons.

Appendix H

Curriculum vitae

Lalith R. J. Liyanage, M.Eng

CONTACT INFORMATION

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Edmonton, Alberta T6G 0T4
Canada.

Telephone: (403) 432-0692 (Home)
(403) 492-3452 (Office)
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E-mail: lliyanag@gpu.srv.ualberta.ca

JOB OBJECTIVE

To work as an Environmental Engineer in a position where opportunity for creative contribution exists and learning and advancement is encouraged

AREAS OF EXPERTISE

- Advanced water and wastewater system design
- Design and analysis of water distribution, wastewater collection systems
- Design and analysis of storm water drainage system
- Advanced statistical design of experiments and data analysis.

OTHER RELATED SKILLS

- Extremely versatile in MS-DOS, Windows, Macintosh operating systems
- Working knowledge and experience in number of computer software related to Environmental Engineering
- Versatile in number of other software, related to statistical analysis, word processing, graphical and database management
- Friendly, team worker.

SUMMARY OF EDUCATIONAL QUALIFICATIONS

Ph.D. Candidate in **Environmental Engineering** , from Department of Civil and Environmental Engineering, Faculty of Graduate Studies and Research, University of Alberta, Edmonton, Alberta, Canada.

Thesis Title: Chlorine dioxide inactivation of *Cryptosporidium parvum* oocysts in water.

Major areas of exposure: Advanced statistical design of experiments, data analysis. Advanced water treatment processes. Treatment plant optimization for controlling both microbial and chemical risks associated with drinking water.

M.Eng. in **Environmental Engineering** (April 1993), from Environmental Engineering Program, The School of Environment, Resources and Development, Asian Institute of Technology, Bangkok, Thailand.

Thesis Title: Kinetics and responses of anaerobic filters to trichloroethylene dosages.

Major areas of exposure: Biological wastewater treatment (aerobic and anaerobic), Biological nutrient removal and industrial wastewater minimization.

B.Sc.(Engg). Hons., First Class in **Civil Engineering** (May 1990), from Department of Civil Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya, Sri Lanka.

Areas of exposure: Fundamentals and applications of civil and environmental engineering, including thermodynamics, hydraulics, chemistry and numerical methods.

SUMMARY OF PUBLICATIONS AND TECHNICAL PRESENTATIONS

Number of publications in refereed journals	4
Number of publications in Conference proceedings	10
Number of published books	4
Number of technical presentations in international audiences	4

SUMMARY OF WORK EXPERIENCE

Special Small Projects Involved.

1. June 1996. Characterization of raw and settled water and evaluation of different chemical disinfectant conditions for inactivation of *Cryptosporidium parvum* oocysts for Austin Water Treatment Plant, Texas.

2. Sept 1997. Evaluation of potential for raw water to be contaminated by *Cryptosporidium* and *Giardia*, evaluation of existing water treatment plant performance with respect to potential of *Cryptosporidium* and *Giardia* removal/inactivation and recommendation of plant upgrade options for Camrose Water Treatment Plant, Camrose, Alberta.

3. Dec 1997. Characterization of raw water with respect to chemical disinfectant behavior and evaluation of inactivation potential of *Cryptosporidium* under simulated plant conditions for Thunder Bay Water Treatment Plant, Ontario.

October 1997 to Date

Research Associate, at the Department of Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta. **Responsibilities include:** Teaching a graduate level course on statistical design of experiments and data analysis. Resource person for AWWARF and USEPA funded research project on evaluation of chemical disinfectants for drinking water. Preparation of project reports and development of a central database for experimental data.

April 1997 to Date

Consultant, to the Associated Engineering Services, Edmonton, Alberta. **Responsibilities include:** Evaluation of drinking water treatment plants for *Cryptosporidium* contamination potential and recommendations for upgrading drinking water treatment plants to control the risks of waterborne parasites.

Sept 1995 to April 1997

Graduate Assistant, Department of Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta, Canada. **Responsibilities include:** Tutoring for the introductory statistic course for fourth year undergraduate students, including laboratory sessions. Supervision of micro-computer lab. Tutoring fourth year undergraduate students for the course dealing with wastewater treatment plant design.

Teaching Assistant, Department of Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta, Canada. **Responsibilities include:** Preparation of lecture notes, teaching and conduct of lab classes for the "water wastewater disinfection" portion of the graduate level course Civ.E 627, Environmental Engineering Laboratories I.

May 1993 to July 1994

Research Associate, Environmental Engineering Program, Asian Institute of Technology, Bangkok, Thailand. **Responsibilities include:** Preparation of lecture materials for low-cost wastewater treatment options for developing countries. Preparation of lecture materials for a course dealing with application of natural systems for the treatment of hazardous waste.

Jan 1992 to April 1993

Student Assistant, Environmental Engineering Division, Asian Institute of Technology, Bangkok, Thailand. **Responsibilities include:** Operation and maintenance of Two Stage Anaerobic Upflow Reactor System for the treatment of swine waste.

June 1990 to July 1991

Civil Engineer (Research), National Building Research Organization, Colombo, Sri Lanka. **Responsibilities include:** Analysis of concrete structural frames using computer software MICROFEAP and STAAD III. Design of concrete structural elements for high-rise building. Construction project management for a high rise building development project.

SCHOLARSHIPS AND AWARDS

Sri Lankan Government Scholarship, As a stipend during Undergraduate program at the University of Peradeniya, Sri Lanka. (Sept 1983- April 1990)

Swedish Government Scholarship, Full scholarship, which covers tuition and a stipend during Masters program at the Asian Institute of Technology, Bangkok, Thailand. (Sept 1991 to April 1993)

University of Alberta Ph. D. Scholarship, Full scholarship which covers tuition and a stipend during first two years of the Ph. D. program at the University of Alberta, Edmonton, Canada. (Sept 1994 to Sept 1996)

Water H Johns Graduate Tuition Scholarship, For the third year tuition fee payment of the Ph.D. program at the University of Alberta, Edmonton, Canada. (Sept-1996 to Sept 1997)

Graduate Student's Association Differential Fee Award, For the third year tuition fee payment of the Ph.D. program at the University of Alberta, Edmonton, Canada. (Sept-1996 to Sept 1997)

MEMBERSHIP OF PROFESSIONAL BODIES

Student member, American Water Works association
Student member, International Ozone Association

SOCIAL ACTIVITIES

Aug 1995 to Dec 1995 **Member**, Access Fund Transitional Committee, University of Alberta Students' Union, University of Alberta, Edmonton, Canada.

May 1992 to Sept 1992 **President**, Sri Lankan Association Asian Institute of Technology, Bangkok, Thailand.

May 1992 to Sept 1992 **Member** of the General Assembly, Student Union, Asian Institute of Technology, Bangkok, Thailand.

May 1992 to Dec 1992 **Committee Member**, Trip and Transportation Committee, Student Union, Asian Institute of Technology, Bangkok, Thailand.

Jan 1987 to April 1990 **Council Member**, Civil Engineering Society, Faculty of Engineering, University of Peradeniya, Peradeniya, Sri Lanka.

PUBLICATIONS

Refereed Journals

1. **Liyanage, L.R.J.**, G.R. Finch, and M. Belosevic. (1997). Effect of Aqueous chlorine and Oxy-chlorine compounds on *Cryptosporidium parvum* oocysts. Environmental Science and Technology, A journal of American Chemical Society. Vol. 31, p 1992-1994.

2. **Liyanage, L.R.J.**, G.R. Finch, and M. Belosevic. (1997). Sequential Disinfection of *Cryptosporidium parvum* by Ozone and Chlorine dioxide. Ozone Science and Engineering, The journal of the International Ozone Association, Vol 19, No.5, p 409-423

3. Belosevic, M., R.A. Guy, R. Taghi-Kilani, N.F. Neumann, L.L. Gyürék, **L.R.J. Liyanage**, P.J. Millard and G.R. Finch. (1997). Nucleic Acid Stains as Indicators of *Cryptosporidium parvum* Oocyst Viability. International Journal for parasitology. Vol. 27, No.7,, pp 787-798

4. Finch, G.R., **L.R.J. Liyanage**, and C.N. Haas. (1997). A review of the efficacy of treatment options for control of waterborne cryptosporidiosis. The Journal of the American Water Works Association. (Submitted for review)

Conference Proceedings

1. Finch, G.R., **L.R.J. Liyanage**, and M. Belosevic. (1995). Effect of Chlorine Dioxide on *Cryptosporidium* and *Giardia*. in Chlorine Dioxide: Drinking Water, Process Water, and Wastewater Issues. Third International Symposium.. New Orleans, LA: American Water Works Association Research Foundation, Chemical Manufacturers Association, and the U. S. Environmental Protection Agency.

2. Taghi-Kilani, R., L.L. Gyürék, **L.R.J. Liyanage**, R.A. Guy, G.R. Finch, and M. Belosevic. (1995). Vital Dye Staining of *Giardia* and *Cryptosporidium*. in Chlorine Dioxide: Drinking Water, Process Water, and Wastewater Issues. Third International Symposium.. New Orleans, LA: American Water Works Association Research Foundation, Chemical Manufacturers Association, and the U. S. Environmental Protection Agency.

3. Finch, G. R., Guyürék, L. L. and **Liyanage, L.R.J**. (1995). Process Design Requirements For Inactivation of *Cryptosporidium* Using Chlorine and Oxy-Chlorine Compounds. Presented at the American Water Works Association, Water Quality Technology Conference, November 12-16, New Orleans, LA.

4. **Liyanage, L.R.J**., L.L. Gyürék, G.R. Finch, and M. Belosevic. (1996). Modeling *Cryptosporidium parvum* Inactivation by Chlorine Dioxide. in 4th Environmental Engineering Specialty Conference. May 29-June 1, Edmonton, Alberta: Canadian Society for Civil Engineering.

5. Gyürék, L.L., G.R. Finch, **L.R.J. Liyanage**, and M. Belosevic. (1996). Disinfection Requirements for Single and Sequential Inactivation of *Cryptosporidium* Using Chlorine Species. in 4th Environmental Engineering Specialty Conference. May 29-June 1, Edmonton, Alberta: Canadian Society for Civil Engineering.

6. **Liyanage, L.R.J**., G.R. Finch, and M. Belosevic. (1996). Sequential Disinfection of *Cryptosporidium parvum* by Ozone and Chlorine dioxide. In Application and Optimization of Ozone for Potable Water Treatment and Other Related Topics. Conference proceedings, The International Ozone Association, Pan American Group. September 8-11, Ottawa, Ontario, Canada.

7. **Liyanage, L.R.J**., Gyürék, L.L., M. Belosevic and G.R. Finch. (1996). Effect of Chlorine dioxide Preconditioning on Inactivation of *Cryptosporidium* by free Chlorine and Monochloramine: Process Design Requiremer.ts. In American Water Works Association, Water Quality Technology Conference, Conference proceedings, November 12-16, Boston, MA.

8. Gyürék, L.L., **Liyanage, L.R.J.**, M. Belosevic and G.R. Finch. (1996). Disinfection of *Cryptosporidium parvum* Oocysts Using Single and Sequential Application of Ozone and Chlorine Species. In American Water Works Association, Water Quality Technology Conference, Conference proceedings, November 12-16, Boston, MA.

9. **Liyanage, L.R.J**. and G.R. Finch. (1997). Synergistic Effects of Sequential Exposure of *Cryptosporidium* oocysts to Chemical Disinfectants. In International Symposium on Waterborne *Cryptosporidium*. Conference proceedings, American Water Works Association, March 2-5, Newport Beach, California.

10.Finch, G. R., Bradbury, J., Guyürék, L. L. and **Liyanage, L.R.J**. (1997). Synergistic Effects of Disinfectants on Waterborne Pathogens. In Environmental Engineering Specialty conference. Conference proceedings, American Society of Civil Engineers and Canadian Society of Civil Engineers, July 1997, Edmonton, Alberta.

Published Books

1. **Liyanage, L.R.J**. (1993). Kinetics and Responses of Anaerobic Filters to Trichloroethylene Dosages. Masters Thesis (April, 1993) Asian Institute of Technology, Bangkok, Thailand.

2. Gordon R. Finch, L.L. Gyürék, **L.R.J. Liyanage** and M. Belosevic. (1997). Effect of Various Disinfection Methods on the Inactivation of *Cryptosporidium*. Published by the American Water Works Association Research Foundation and American Water Works Association.

3. **Liyanage, L.R.J**. (1997). Chlorine dioxide inactivation of *Cryptosporidium parvum* oocysts in Water. Ph.D. Thesis (Sept, 1997) University of Alberta, Edmonton, Canada. (In preparation).

4. Belosevic, M., R. Taghi-Kilani, R.A. Guy, N.F. Neumann, G.R. Finch, L.L. Gyürék, **L.R.J. Liyanage**. (1997). Vital Dye Staining of *Giardia* and *Cryptosporidium*. Published by the American Water Works Association Research Foundation and American Water Works Association

TECHNICAL PRESENTATIONS

1. Inactivation of *Cryptosporidium parvum* by chlorine dioxide: a critical look at the CT values proposed under the U. S. EPA Enhanced Surface Water Treatment Rule. Presented at the Conference, **The Future of Our Environment: Research at the University of Alberta. 19 April 1996, University of Alberta, Edmonton, Canada.**

2. Sequential Disinfection of *Cryptosporidium* parvum by Ozone and Chlorine dioxide. Presented at the Conference, Application and Optimization of Ozone for Potable Water Treatment and Other Related Topics. **The International Ozone Association, Pan American Group. September 8-11, Ottawa, Ontario, Canada.**

3. Effect of Chlorine dioxide Pre conditioning on Inactivation of *Cryptosporidium* by free Chlorine and Monochloramine: Process Design Requirements. **Presented at the American Water Works Association, Water Quality Technology Conference, November 12-16, Boston, MA. United States.**

4. Synergistic Effects of Disinfectants on Waterborne Pathogens. Presented at the Environmental Engineering Specialty conference. **American Society of Civil Engineers and Canadian Society of Civil Engineers, July 1997, Edmonton, Alberta.**

LISTING OF COURSE WORKS COMPLETED

Undergraduate level (Civil Engineering major)

Third year

Mathematics
Theory of Structures and Highway Engineering
Strength of Materials and Material Science
Surveying and Geology
Mechanics of Fluids and Hydrology
Electrical Engineering

Fourth year

Industrial Engineering
Geotechnics
Theory of Structures and Strength of Materials
Irrigation and Public Health Engineering
Surveying
Concrete Structures and Technology
Environmental Engineering

Masters Level (Environmental Engineering Major)

Physico-Chemical Processes
Environmental Chemistry and Laboratory
Biological Processes
Environmental Science and Management
Environmental Health and Sanitation
Wastewater Engineering Design
Advanced Water and Wastewater Treatment Processes
Bioprocess Technology

Organic Waste Recycling
Water Engineering Design
Modeling of Groundwater Systems
Groundwater Pollution and Transport Modeling

Doctoral level (Environmental Engineering Major)

Process Dynamics and Control
Design of Civil Engineering Experiments
Soil Environment Chemistry
Environmental Engineering Measurement II
Numerical Methods in Hydraulics
Experimental Parasitology